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Main Menu | Search Form | Posting Counts | Show S Numbers | Edit S Numbers | Preferences | Cases

Search Results -

Terms	Documents
pCIS25DTR	3

Database: US Patents Full-Text Database; US Pre-Grant Publication Full-Text Database; JPO Abstracts Database; EPO Abstracts Database; Derwent World Patents Index; IBM Technical Disclosure Bulletins

Search: L8

Refine Search | Recall Text | Clear

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DATE: Monday, December 08, 2003 Printable Copy Create Case

Set Name side by side	Query	Hit Count	Set Name result set
DB=USPT; PLUR=YES; OP=OR			
L8	pCIS25DTR	3	L8
L7	cell line production	2341619	L7
L6	cell adj2 vector adj2 marker adj2 promoter	0	L6
L5	l2 and L4	426002	L5
L4	L3 and l1	426002	L4
L3	HKB11 cells	426002	L3
L2	293S cells	518071	L2
L1	cell production adj2 factor VIII	515096	L1

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☐ 1. Document ID: US 6358703 B1

L8: Entry 1 of 3 File: USPT Mar 19, 2002

US-PAT-NO: 6358703
DOCUMENT-IDENTIFIER: US 6358703 B1

TITLE: Expression system for factor VIII

DATE-ISSUED: March 19, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Cho; Myung-Sam	Pinole	CA		
Chan; Sham-Yuen	El Sobrante	CA		
Kelsey; William	Alameda	CA		
Yee; Helena	San Francisco	CA		

US-CL-CURRENT: 435/69.1; 435/325, 435/346, 435/366, 435/372, 435/69.6, 530/350, 530/383

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Claims | KWIC | Draw Desc | Image

☐ 2. Document ID: US 6180108 B1

L8: Entry 2 of 3 File: USPT Jan 30, 2001

US-PAT-NO: 6180108
DOCUMENT-IDENTIFIER: US 6180108 B1

TITLE: Vectors having terminal repeat sequence of Epstein-Barr virus

DATE-ISSUED: January 30, 2001

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Cho; Myung-Sam	Pinole	CA		
Chan; Sham-Yuen	El Sobrante	CA		

US-CL-CURRENT: 424/199.1; 424/204.1, 435/235.1, 435/239, 435/320.1, 435/91.1, 435/91.33

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Claims | KWIC | Draw Desc | Image

☐ 3. Document ID: US 6136599 A

L8: Entry 3 of 3 File: USPT Oct 24, 2000

US-PAT-NO: 6136599
DOCUMENT-IDENTIFIER: US 6136599 A

TITLE: Human hybrid host cell for mammalian gene expression

DATE-ISSUED: October 24, 2000

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Cho; Myung-Sam	Pinole	CA		

US-CL-CURRENT: 435/325; 435/326, 435/335, 435/346

Terms	Documents
pCIS25DTR	3

L8: Entry 1 of 3 File: USPT Mar 19, 2002

DOCUMENT-IDENTIFIER: US 6358703 B1
TITLE: Expression system for factor VIII

Drawing Description Text (4):
FIG. 3. Plasmid map of pCIS25DTR.

Detailed Description Text (5):
The sequence of the B-domain deleted (BDD) FVIII is shown in FIG. 1. The 90-kD and 80-kD chains were linked by a linker consisting of 14 amino acids. See Chan, S.-Y., "Production of Recombinant Factor VIII in the Presence of Liposome-like Substances of Mixed Composition," U.S. patent application Ser. No. 08/634,001, filed Apr. 16, 1996. The expression vector for BDD-FVIII was made using standard recombinant DNA techniques. The structure of the expression vector (pCIS25DTR) is shown in FIG. 3. The vector includes a transcriptional unit for BDD-FVIII and a selectable marker, dihydrofolate reductase (dhfr). In addition a terminal repeat sequence from Epstein-Barr virus, which shows enhanced drug selection ratio, (FIG. 2) was inserted into the vector to increase the integration efficiency. The vector is essentially a construct of a vector (deposited ATCC 98879) which has been engineered to include a transcriptional unit corresponding to the sequence shown in FIG. 1. Further information about the terminal repeat sequence can be found in the related patent application, incorporated herein by reference, to Cho and Chan Ser. No. 09/209,915, "Terminal repeat sequence of Epstein-Barr virus enhances drug selection ratio," filed on the same day as the current application.

Detailed Description Text (9):
Thirty micrograms of pCIS25DTR DNA was transferred into HKB11 (ATCC deposit no. CRL 12568--a hybrid of 293S cells and human Burkitt's lymphoma cells, see U.S. patent application to Cho et al. filed on the same day as the current application Ser. No. 09/209,920, incorporated herein by reference) cells by electroporation set at 300 volts and 300 micro farads (BTX Electro cell Manipulator 600) using a 2 mm cuvette (BTX part #620). In comparative experiments done to parallel work with the HKB11 cells, CHO (Chinese hamster ovary) and 293S (human embryonic kidney) cells were transfected using a cationic lipid reagent DMRIE-C (Life Technologies, Gaithersburg, Md.) according to a protocol provided by the Life Technologies. Amplification of transfected cells was done with increasing methotrexate (MTX) concentrations (100 nM, 200 nM, 400 nM, and 800 nM) at 1.times.10.sup.6 cells per 96 well plate in a MTX-selection medium lacking hypoxanthine and thymidine (DME/F12 media without hypoxanthine and thymidine plus 5% dialyzed fetal bovine serum from Hyclone, Logan, Utah). MTX resistant cells were scored for growth, and secretion of the BDD-FVIII was screened using a Coatest.RTM. factor VIII kit about 2-3 weeks post-transfection. The cultivation of cells were done at 37.degree. C. in a humidified 5% CO.sub.2 incubator.

Detailed Description Text (13):
As summarized in FIG. 4(a), the initial population 1C10 was derived from the HKB cells transfected with pCIS25DTR after amplification with 400 nM MTX in the selection medium with 5% FBS. One of the first single cell clones (SCCs), 10A8, derived from 1C10 by a LDC using a selection medium supplemented with 5% FBS was adapted in serum-free medium supplemented with Plasmanate.RTM. HPP fraction. Unexpectedly, 10A8 showed extremely increased levels of rFVIII production at this stage (FIG. 4b). Therefore, we did a second LDC using the medium supplemented with Plasmanate.RTM. HPP fraction. The productivity of SCCs (e.g. 20B8) derived from the second LDC was similar with Plasmanate.RTM. HPP fraction-adapted 10A8. 20B8 showed

higher levels of BDD-FVIII than original 10A8 derived from the first LDC in serum-containing medium. Finally, 20B8 was adapted to growth in plasma protein-free (PPF) medium. Samples of 20B8 were deposited at the American Type Culture Collection (Manassas, Va.) (ATCC deposit no. CRL-1 2582).

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L8: Entry 3 of 3 File: USPT Oct 24, 2000

DOCUMENT-IDENTIFIER: US 6136599 A
TITLE: Human hybrid host cell for mammalian gene expression

Drawing Description Text (3):
FIG. 2 shows physical maps of expression vectors mentioned in the text. All plasmids are constructed based on a pBR322 backbone and contain a dhfr expression unit. All genes coding proteins of interest are under the regulation of CMV enhancer/promoter (CMVe/p); 5'intron (MIS or CIS) was positioned at the 5'end of the genes, except BZLF1. Poly A signal region was indicated as pA. Both plasmids, pSH157 and pCIS25DTR, contain a sequence of EBV-TR (402 bp).

Detailed Description Text (5):
All expression vectors used in this report were basically pBR322-based plasmid with function dhfr gene expression segement. Physical maps of expression vectors are described in FIG. 2. Plasmids, pSH157 and pCIS25DTR, have also terminal repeat sequence of Epstein-Barr virus (EBV-TR). See patent application to Cho and Chan designated MSB-7254, "Terminal repeat sequence of Epstein-Barr virus enhances drug selection ratio," for the EBV-TR sequence. The vector pSH13 1, which has been deposited with the American Type Culture Collection, ATCC 98879, may be used to generate expression vectors for a chosen protein as described in Cho and Chan (MSB-7254, supra.)

Detailed Description Text (34):
HKB13 transfected with pSS125 after amplification in 50 nM MTX and limiting dilution cloning without MTX, (iii) truncated rFVIII (BDD-FVIII) secreting single cell clones (5-10 .mu.U/c/d, in serum-free condition) derived from HKB11 cells transfected with pCIS25DTR after amplification (400 nM MTX) and limiting dilution cloning without MTX, and (iv) IL-4 derivative (IL-4 selective agonist, IL-4SA; mutated two positions of amino acid, T13D and R121E) secreting clones (5 pg/c/d) derived from HKB11 transfected with pSH157 after MTX amplification. The IL-4SA secreting HKB clone, 1G2, (deposited on May 19, 1999 with American Type Culture Collection, 10801 University Blvd., Manassas, Va. 20110, and granted accession number PTA-87) was used for relatively quick production of small amounts of protein (gram quantity). See FIG. 2 for the physical map of the expression vectors mentioned above.

L8: Entry 2 of 3 File: USPT Jan 30, 2001

DOCUMENT-IDENTIFIER: US 6180108 B1
TITLE: Vectors having terminal repeat sequence of Epstein-Barr virus

Brief Summary Text (13):
The expression vectors of this invention include an EBV-TR sequence and a selectable marker, such as dihydrofolate reductase (dhfr). The preferred EBV-TR sequence is a 402 bp sequence (given in FIG. 1) which includes the core part of the TRBP-binding region from an immortalized lymphoblastoid cell line 6F 11. In a preferred embodiment, the mammalian gene expression vector comprises a CMV enhancer and promoter, an intronic sequence (MIS, as described in U.S. Pat. No. 5,854,021 to Cho et al.) derived from Epstein-Barr virus, a unique restriction enzyme site Hpal to allow for insertion of a protein coding sequence, and a poly A region plus the plasmid backbone with a drug selection marker and the EBV-TR sequence indicated in FIG. 1. This vector is denoted pSH131 (see FIG. 2). This vector is used to introduce the appropriate DNA coding sequence of the protein of interest into mammalian cells to stabilize the protein expression in a long term culture in a serum-free medium. In one preferred embodiment, the sequence for an IL-4 mutein was cloned into pSH131 and the resulting vector is pSH135. The EBV-TR sequence was also directly linked to pCIS25D (vector for expressing B-domain deleted rFVIII, designated BDD-FVIII) and resulting vector is pCIS25DTR.

Detailed Description Text (6):
The PCR product of the EBV-TR sequence was also inserted into the SalI site of pCIS25D, which is an expression vector coding for B-domain deleted factor VIII (BDD-FVIII). The resulting plasmid is pCIS25DTR. All four expression vectors, pSH134 and pSH135, pCIS25D, and pCIS25DTR, have the same functional dhfr gene. See FIG. 2 for the maps.

Detailed Description Text (13):
Two weeks after seeding the cells in selection medium, pSH135 (with EBV-TR) transfected cells showed an approximately 10 fold higher selection ratio than those transfected with pSH134 (without EBV-TR), although EBV-TR showed no enhancing function on IL-4 expression (FIG. 3). CHO (dhfr-) cells were also transfected with 5 .mu.g of pCIS25D and 5 .mu.g of pCIS25DTR using DMRIE-C reagent. Cells were selected under the same conditions as described above. Cells transfected with pCIS25DTR showed about 3-fold to about 16-fold higher selection ratio than those transfected with pCIS25D (Table 1). These results indicate that this sequence of EBV-TR in the expression vector can be used for increased integration of the vector in vivo for gene therapy.

Detailed Description Text (19):
One of the CHO clones secreting BDD-FVIII, which was derived from pCIS25DTR transfected CHO cells as described in Example 2, was tested for its production stability under the absence of the selection drug (MTX). This clone continued to secrete BDD-FVIII during a six month period of growth in a medium lacking MTX. All single cell clones derived from this clone were also secretion positive. These results indicate that the integration of the vectors containing an EBV-TR sequence is a stable integration.

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L9: Entry 1 of 3 File: USPT Mar 19, 2002

DOCUMENT-IDENTIFIER: US 6358703 B1
TITLE: Expression system for factor VIII

Abstract Text (1):
This invention describes a protein-free production process for proteins having factor VIII procoagulant activity. The process includes the derivation of stable human cell clones with high productivity for B-domain deleted Factor VIII, and (2) the adaptation of cells to grow in a medium free of plasma-derived proteins.

Parent Case Text (2):
The application to Cho designated Ser. No. 09/209,920, filed Dec. 10, 1998, now U.S. Pat. No. 6,136,599, title "Human hybrid host cell for mammalian gene expression," and the application to Cho and Chan designated Ser. No. 09/209915, filed Dec. 10, 1998, now U.S. Pat. No. 6,180,108, titled "Terminal repeat sequence of Epstein-Barr virus enhances drug selection ratio," contain related subject matter.

Brief Summary Text (3):
The present invention relates to an improved production method for factor VIII and its derivatives. The method relates generally to vector construction, transfection, and selection of cell lines with enhanced productivity under protein-free conditions. In particular, this invention relates to a process for preparing a protein with factor VIII procoagulant activity on an industrial scale.

Brief Summary Text (5):
Human factor VIII is a trace plasma glycoprotein involved as a cofactor in the activation of factor X and factor IXa. Inherited deficiency of factor VIII results in the X-linked bleeding disorder hemophilia A which can be treated successfully with purified factor VIII. The replacement therapy of hemophilia A has evolved from the use of plasma-derived factor VIII to the use of recombinant factor VIII obtained by cloning and expressing the factor VIII cDNA in mammalian cells. (Wood et al., 1984, Nature 312: 330).

Brief Summary Text (6):
Factor VIII has a domain organization of A1-A2-B-A3-C1-C2 and is synthesized as a single chain polypeptide of 2351 amino acids, from which a 19-amino acid signal peptide is cleaved upon translocation into the lumen of the endoplasmic reticulum. Due to the fact that factor VIII is heavily glycosylated, high-level expression (>0.2 pg/c/d) of factor VIII has been difficult to achieve (Lind et al., 1995, Eur J Biochem. 232: 19-27; Kaufman et al., 1989, Mol Cell Biol. 9: 1233-1242). Expression of factor VIII in mammalian cells is typically 2-3 orders of magnitude lower than that observed with other genes using similar vectors and approaches. The productivity of production cell lines for factor VIII has been in the range of 0.5-1 .mu.U/c/d (0.1-0.2 pg/c/d).

Brief Summary Text (7):
It has been demonstrated that the B-domain of factor VIII is dispensable for procoagulant activity. Using truncated variants of factor VIII, improved expression of factor VIII in mammalian cells has been reported by various groups (Lind et al., 1995, Eur J Biochem 232: 19-27; Tajima et al., 1990, Proc 6.sup.th Int Symp H.T. p.51-63; U.S. Pat. No. 5,661,008 to Almstedt, 1997). However, the expression level of the factor VIII variants remained below 1 pg/c/d from a stable cell clone.

Brief Summary Text (9):

We have now discovered (i) a method which derives cell lines with extremely high productivity of proteins having factor VIII procoagulant activity, and (ii) a plasma protein-free production process for proteins having factor VIII procoagulant activity.

Brief Summary Text (10):
A process for the production of proteins having factor VIII procoagulant activity at the industrial scale is disclosed. Using a newly created cell host, cell clones with specific productivities in the range of 2-4 pg/cell/day (10-20 .mu.U/c/d) were derived. Under serum-free conditions, one clone has sustained a daily productivity of 2-4 pg/c/d. Clones with this high level of productivity are able to produce 3-4 million units per day in a 15-liter perfusion fermenter. One unit of factor VIII activity is by definition the activity present in one milliliter of plasma. One pg of factor VIII is generally equivalent to about 5 .mu.U of FVIII activity.

Brief Summary Text (12):
A high level of expression of a protein having factor VIII procoagulant activity means at least about 2 .mu.U/c/d, or more preferably at least about 4 .mu.U/c/d, or most preferably at least about 5 .mu.U/c/d, of factor VIII activity if grown in plasma derived protein-free medium, or at least about 4 .mu.U/c/d, or more preferably at least about 8 .mu.U/c/d, or most preferably at least about 10 .mu.U/c/d, of factor VIII activity if grown in medium supplemented with plasma derived protein. When the protein expressed is BDD-FVIII, cell lines having specific productivities up to about 15 .mu.U/c/d, more preferably up to about 20 .mu.U/c/d may be obtained by the method described herein.

Brief Summary Text (13):
As used herein to describe the origin of cell lines, "derived from" is intended to include, but not be limited to, normal mitotic cell division and processes such as transfections, cell fusions, or other genetic engineering techniques used to alter cells or produce cells with new properties.

Drawing Description Text (4):
FIG. 3. Plasmid map of pCIS25DTR.

Detailed Description Text (3):
The activity of factor VIII derivatives obtained from recombinant gene expression in methotrexate (MTX)-resistant cell populations was measured by a chromogenic assay. Activity was quantitated using Coatest.RTM. factor VIII:C/4 kit (Cromogenix, Molndal, Sweden) according to manufacturer's instructions. A U.S. standard anti-hemophilic factor (factor VIII) known as MEGA 1 (Office of Biologics Research and Review, Bethesda, Md.) was used as the standard of measurement in this assay. See Barrowcliffe, 1993, Thromb Haem 70: 876.

Detailed Description Text (5):
The sequence of the B-domain deleted (BDD) FVIII is shown in FIG. 1. The 90-kD and 80-kD chains were linked by a linker consisting of 14 amino acids. See Chan, S.-Y., "Production of Recombinant Factor VIII in the Presence of Liposome-like Substances of Mixed Composition," U.S. patent application Ser. No. 08/634,001, filed Apr. 16, 1996. The expression vector for BDD-FVIII was made using standard recombinant DNA techniques. The structure of the expression vector (pCIS25DTR) is shown in FIG. 3. The vector includes a transcriptional unit for BDD-FVIII and a selectable marker, dihydrofolate reductase (dhfr). In addition a terminal repeat sequence from Epstein-Barr virus, which shows enhanced drug selection ratio, (FIG. 2) was inserted into the vector to increase the integration efficiency. The vector is essentially a construct of a vector (deposited ATCC 98879) which has been engineered to include a transcriptional unit corresponding to the sequence shown in FIG. 1. Further information about the terminal repeat sequence can be found in the related patent application, incorporated herein by reference, to Cho and Chan Ser. No. 09/209,915, "Terminal repeat sequence of Epstein-Barr virus enhances drug selection ratio," filed on the same day as the current application.

Detailed Description Text (6):
Similar vectors can be constructed and used by those having skill in the art to obtain cells expressing proteins having factor VIII procoagulant activity. For

example, coding sequences coding for known variants of factor VIII which retain procoagulant activity can be substituted for the BDD-FVIII coding sequence. Also, instead of dhfr, other selectable markers can be used, such as glutamine synthetase (gs) or multidrug-resistance gene (mdr). The choice of a selection agent must be made accordingly, as is known in the art, i.e. for dhfr, the preferred slection agent is methotrexate, for gs the preferred selection agent is methionine sulfoximine, and for mdr the preferred selection agent is colchicine.

Detailed Description Text (8):
Derivation of Cell Lines Expressing BDD-FVIII: Transfection, Drug Selection and Gene Amplification

Detailed Description Text (9):
Thirty micrograms of pCIS25DTR DNA was transferred into HKB11 (ATCC deposit no. CRL 12568--a hybrid of 293S cells and human Burkitt's lymphoma cells, see U.S. patent application to Cho et al. filed on the same day as the current application Ser. No. 09/209,920, incorporated herein by reference) cells by electroporation set at 300 volts and 300 micro farads (BTX Electro cell Manipulator 600) using a 2 mm cuvette (BTX part #620). In comparative experiments done to parallel work with the HKB11 cells, CHO (Chinese hamster ovary) and 293S (human embryonic kidney) cells were transfected using a cationic lipid reagent DMRIE-C (Life Technologies, Gaithersburg, Md.) according to a protocol provided by the Life Technologies. Amplification of transfected cells was done with increasing methotrexate (MTX) concentrations (100 nM, 200 nM, 400 nM, and 800 nM) at 1.times.10.sup.6 cells per 96 well plate in a MTX-selection medium lacking hypoxanthine and thymidine (DME/F12 media without hypoxanthine and thymidine plus 5% dialyzed fetal bovine serum from Hyclone, Logan, Utah). MTX resistant cells were scored for growth, and secretion of the BDD-FVIII was screened using a Coatest.RTM. factor VIII kit about 2-3 weeks post-transfection. The cultivation of cells were done at 37.degree. C. in a humidified 5% CO.sub.2 incubator.

Detailed Description Text (11):
Single cell clones (SCC) were derived by limiting dilution cloning (LDC) of high producing populations in 96 well plates under serum-free conditions. Cells were seeded at 1-10 cells per well in DME/F12 media supplemented with Humulin.RTM. recombinant insulin (Lilly, Indianapolis, Ind.) at 10 .mu.g/ml, 10X essential amino acids (Life Technology, Gaithersburg, Md.), and Plasmanate.RTM. human plasma protein fraction (Bayer, Clayton, N.C.). Plasmanate.RTM. human plasma protein (HPP) fraction contains human albumin (88%) and various globulins (12%). The clones were screened for BDD-FVIII productivity using the Coatest.RTM. factor VIII kits. The highest producing clones were selected for stability evaluation in shake flasks. For HKB cells, the first round LDC was performed using selection medium supplemented with 5% dialyzed FBS. The second round LDC was done in serum-free but Plasmanate.RTM. HPP fraction-containing medium using the first SCC adapted in serum-free medium supplemented with Plasmanate.RTM. HPP fraction.

Detailed Description Text (13):
As summarized in FIG. 4(a), the initial population 1C10 was derived from the HKB cells transfected with pCIS25DTR after amplification with 400 nM MTX in the selection medium with 5% FBS. One of the first single cell clones (SCCs), 10A8, derived from 1C10 by a LDC using a selection medium supplemented with 5% FBS was adapted in serum-free medium supplemented with Plasmanate.RTM. HPP fraction. Unexpectedly, 10A8 showed extremely increased levels of rFVIII production at this stage (FIG. 4b). Therefore, we did a second LDC using the medium supplemented with Plasmanate.RTM. HPP fraction. The productivity of SCCs (e.g. 20B8) derived from the second LDC was similar with Plasmanate.RTM. HPP fraction-adapted 10A8. 20B8 showed higher levels of BDD-FVIII than original 10A8 derived from the first LDC in serum-containing medium. Finally, 20B8 was adapted to growth in plasma protein-free (PPF) medium. Samples of 20B8 were deposited at the American Type Culture Collection (Manassas, Va.) (ATCC deposit no. CRL-1 2582).

Detailed Description Text (14):
As shown in Table 1, HKB clones exhibit superior productivity for BDD-FVIII. A 10-20 fold increase in productivity was observed in HKB cells when compared to clones derived from transfected CHO and 293S cells. HKB cells, which do not form large

aggregates of cells when grown in suspension culture, are preferred cells for the expression of proteins having factor VIII procoagulant activity.

Detailed Description Text (16):
HKB clones that have been adapted to grow as serum-free suspension cultures were further weaned of plasma protein supplements. The weaning was done in sterile polycarbonate shake flasks (Corning, Corning, N.Y.) at a cell density of about 0.5.times.10.sup.6 cells/ml using plasma derived protein free medium. The plasma protein free (PPF) medium was DME/F12 medium supplemented with pluronic F68 (0.1%), CuSO.sub.4 (50 nM), and FeSO.sub.4 /EDTA (50 .mu.M). Complete medium exchange was done every 48 hours and the shake flasks were re-seeded at 0.5.times.10.sup.6 cells/ml.

Detailed Description Text (18):
The productivity of clone 20B8 was evaluated in a 15-liter perfusion fermenter. The fermenter was seeded with clone 20B8 cells at a density of about 3.times.106 cells/ml. The fermenter was perfused at a rate of 4 volumes per day with the serum-free production medium as described in the preceding paragraph. A final cell density of 2.times.10.sup.7 cells/ml was sustained throughout the evaluation period (45 days). As shown in FIG. 5, during the first 4 weeks of fermentation, clone 20B8 was perfused with the serumfree production medium supplemented with Plasmanate.RTM. HPP fraction and was able to sustain high productivity. From day 28 to the end of the fermentation run, the cells were perfused with the same serumfree production medium but without Plasmanate.RTM. HPP fraction. As shown in FIG. 5, the cells continued to produce high levels of FVIII in a plasma derived protein-free environment. "Plasma derived protein-free" means that essentially no proteins isolated from plasma have been added to the medium.

Detailed Description Text (20):
The derivation of HKB cells provides a protein-free production system to produce not only BDD-FVIII but other therapeutic proteins as well. Proteins produced from HKB cells have human glycosylation patterns which may improve the half-life of certain glycoproteins in vivo. These cells should also be useful for the production of adenovirus and adeno-associated virus strains that have been designed for gene therapy purposes.

Detailed Description Paragraph Table (1):
TABLE 1 Expression of FVIII and BDD-FVIII in human and rodent cell lines Specific Productivity (.mu.U/c/d)* FVIII Derivatives BHK 293s CHO HKB Full length FVIII 0.45 1.2 0.5 1.0 BDD-FVIII ND 2.5 1.0 20 *Average of 5 high producing clones (in serum-free media) ND = Not done

Other Reference Publication (1):
Pu et al. Rapid Establishment of High-Producing Cell Lines Using Dicistronic Vectors with Glutamine Synthetase as teh Selection Marker. Mol. Biotechnol. 10: 17-25, 1998.*

Other Reference Publication (2):
Kane, S.E. Selection of Transfected Cells and Coamplification of Transfected Genes. Methods Mol. Biol. 62: 359-367, 1997.*

Other Reference Publication (3):
Bebbington et al. High-Level Expression of a Recombinant Antibody from Myeloma Cells using a Glutamine Synthetase Gene as an Amplifiable Selectable Marker. Bio/Technol. 10: 169-175, Feb. 1992.

CLAIMS:

1. A method of producing and isolating a protein having factor VIII activity comprising growing cells designated by the American Type Culture Collection as CRL-12568 which include a sequence coding for the protein operably linked to a promoter, the growing being under conditions sufficient to express the protein and isolating the protein.

3. The method of claim 1 wherein the protein is expressed at a level of at least 2

.mu.U/c/d when the cells are grown in a plasma derived protein-free medium.

6. A human cell line obtained from cells designated by the American Type Culture Collection as CRL-12568 which express a protein having factor VIII activity.

7. The human cell line of claim 6 which expresses B-domain deleted factor VIII.

8. A cell line designated by the American Type Culture Collection as CRL-12582.

L9: Entry 2 of 3 File: USPT Jan 30, 2001

DOCUMENT-IDENTIFIER: US 6180108 B1
TITLE: Vectors having terminal repeat sequence of Epstein-Barr virus

Abstract Text (1):
The use of a unique terminal repeat sequence derived from Epstein-Barr virus to improve the integration frequency of heterologous expression vectors in transfected cells is described. The vectors can be used in a process for deriving high producing cell lines.

Brief Summary Text (2):
The application to Cho designated MSB-7241, "Human hybrid host cell for mammalian gene expression," and the application to Cho et al. designated MSB-7255, "Expression system for factor VIII," contain related subject matter. Both applications were filed on the same day as the current application and are incorporated herein by reference.

Brief Summary Text (5):
This invention relates generally to the production of biologically active proteins from genetically engineered mammalian cell lines. Specifically, the invention is concerned with a novel expression vector containing an Epstein-Barr virus terminal repeat sequence which enhances integration of expression vectors into the genomic DNA in host mammalian cell lines.

Brief Summary Text (8):
Random, nonhomologous integration of input DNA into the host cell genome occurs more than 100 times more frequently than targeted homologous recombination (Thomas et al., 1987, Cell 51: 503-512). However, homologous recombination using hotspot, e.g. hypervariable minisatellite DNA, was shown to occur more frequently than random recombination between two defective plasmids in mammalian cells (Wahls et al., 1990, Cell 60: 95-103).

Brief Summary Text (10):
The terminal repeat binding protein binds to G-rich regions of terminal repeats of Epstein-Barr virus (EBV-TR). EBV-TR takes part in processing and packaging of virion DNA (Zimmermann et al., 1995, J Virol 69: 3147-3155). The EBV-TRs are involved in the integration into chromosomal DNA (Matsuo et al., 1984, Science 226: 1322-1325) and in the circularization event of the genome after infection. These sequences are the essential elements for cleavage and packaging of the EBV virion DNA (Hammerschmidt et al., 1989, Nature (London) 340: 393-397; Zimmermann et al. J Virol, 1995, 69: 3147-3155). These data indicate the important role of the EBV-TR sequence in the recombination events. Therefore, we tested EBV-TR for integration events in deriving clones from the transfected cells.

Brief Summary Text (12):
We have now discovered that cells transfected with an expression vector containing a selectable marker and an EBV-TR sequence show a five to ten fold increase in the number of cells resistant to the selection agent as compared to cells transfected with the same expression vector without an EBV-TR sequence under the same selection conditions. The higher survival ratios under drug selection indicate that the vectors with EBV-TR may enhance the integration frequency of vectors into genomic DNA.

Brief Summary Text (13):

The expression vectors of this invention include an EBV-TR sequence and a selectable marker, such as dihydrofolate reductase (dhfr). The preferred EBV-TR sequence is a 402 bp sequence (given in FIG. 1) which includes the core part of the TRBP-binding region from an immortalized lymphoblastoid cell line 6F 11. In a preferred embodiment, the mammalian gene expression vector comprises a CMV enhancer and promoter, an intronic sequence (MIS, as described in U.S. Pat. No. 5,854,021 to Cho et al.) derived from Epstein-Barr virus, a unique restriction enzyme site Hpal to allow for insertion of a protein coding sequence, and a poly A region plus the plasmid backbone with a drug selection marker and the EBV-TR sequence indicated in FIG. 1. This vector is denoted pSH131 (see FIG. 2). This vector is used to introduce the appropriate DNA coding sequence of the protein of interest into mammalian cells to stabilize the protein expression in a long term culture in a serum-free medium. In one preferred embodiment, the sequence for an IL-4 mutein was cloned into pSH131 and the resulting vector is pSH135. The EBV-TR sequence was also directly linked to pCIS25D (vector for expressing B-domain deleted rFVIII, designated BDD-FVIII) and resulting vector is pCIS25DTR.

Brief Summary Text (15):
The cell host to be transfected can be any mammalian cells. Cell lines that are known to accept the integration of selection genes into their chromosomal DNA are optimal; for example, human embryonic kidney cells (e.g. 293S cells), human hybrid of 293S and B-cell origin (e.g. HKB11; ATCC deposit no. CRL 12568, see U.S. Patent application to Cho designated MSB-7241, "Human hybrid host cell for mammalian gene expression," filed on the same day as the current application and incorporated herein by reference), chinese hamster ovary (CHO), baby hamster kidney (BHK-21), mouse myeloma, and human B-cells.

Brief Summary Text (16):
As one working example, we show that CHO (dhfr-) cells transfected with an expression vector containing dhfr and an EBV-TR sequence showed about a five to ten fold increase in the number of methotrexate (MTX) resistant cells as compared to cells transfected with the same expression vector without EBV-TR under the same selection conditions.

Brief Summary Text (17):
As used herein, serum-free conditions means conditions in which cell growth occurs in media lacking any added serum.

Drawing Description Text (4):
FIG. 3 shows the effect of the EBV-TR sequence on the expression of an IL-4 mutein from pSH134 and pSH135 in transient transfection assays repeated four times using CHO and HKB cells.

Detailed Description Text (3):
A 381 bp fragment of the EBV-TR sequence described in FIG. 1, encompassing DNA sequence from 170,476 to 170,856 of B95/8 EBV sequencing data (P. J. Farrell, "Epstein-Barr Virus Genome," in Advanced Viral Oncology; edited by G. Klein; Ravens Press, Ltd.: New York 1989, pp 103-132) was made by polymerase chain reaction (PCR) from a template DNA prepared from 6F11 cells (ATCC CRL9562) a lymphoblastoid cell line which was immortalized by EBV. Two primers (5'-GGCAATGGAGCGTGACGAAG-3' and 5'-CTCATCACGGTCACGCATGG-3', fragments derived from SEQ ID:1) were made to amplify the 381 bp fragment of the EBV-TR sequence in 6F11 cell DNA. The PCR products were phosphorylated and linked to the expression vector pSM97 after removing a 553 bp fragment excised by the restriction endonuclease Nael. The resulting vector was pSH131 (FIG. 2), which has been deposited with the American Type Culture Collection, ATCC 98879.

Detailed Description Text (4):
DNA sequencing data of the EVB-TR sequence in pSH131 (402 bp) was larger than the expected size (381 bp). The main difference is a repeat of 11-bp (GGCGGGTCATG) consisting of 4 bp from a 9 bp element (GTGTTGGCG)and 7 bp from a 10 bp element (GGTCATGGGG). Both ends of EBV DNA molecule in EBV-TR described by Zimmermann et al. (1996, J Virol 69: 3147-3155) consists of a 9 bp element (GTGTTGGCG) and a 11 bp element (GGGTCATGGGG) (all fragments derived from SEQ ID NO: 1). The 11 bp element in pSH131 lacked 1 bp; thus we observed only 10 bp. The reason for the observed

repeat of the 11 bp element might be that the EBV-TR sequence in pSH131 (402 bp) was made using 6F11 DNA, not B95/8 DNA. 6F 11 cells are immortalized by an EBV and have a concatenated form of EBV-DNA (Cho and Tran, 1993, Virology 194: 838-842), while B95/8 EBV is an infecting virus. Therefore, the EBV-TR sequence in pSH131 (402 bp) was derived from this concatenated EVB-DNA.

Detailed Description Text (6):
The PCR product of the EBV-TR sequence was also inserted into the SalI site of pCIS25D, which is an expression vector coding for B-domain deleted factor VIII (BDD-FVIII). The resulting plasmid is pCIS25DTR. All four expression vectors, pSH134 and pSH135, pCIS25D, and pCIS25DTR, have the same functional dhfr gene. See FIG. 2 for the maps.

Detailed Description Text (9):
Two million CHO (dhfr-negative) and HKB (a human-human hybrid cell line; ATCC CRL-12568) cells were separately transfected with 5 ug of plasmid DNA (pSH134 and pSH135) in a 6-well plate using cationic liposome DMRIE-C reagent (Life Technologies, Rockville, Md.) according to the protocol provided. Two or three days after transfection of CHO and HKB cells with both expression vectors, the supernatants were tested for expression of the IL-4dm by an ELISA. As shown in FIG. 3, expression levels of IL-4dm from pSH134 were very similar with those from pSH135 from two different transfectants in repeated transient transfection assays. These results show that EBV-TR has no effects on the expression of the IL-4dm reporter gene. These results indicate that EBV-TR might not have any enhancing function on the gene expression in the vectors, e.g. dhfr. This implies that the presence of the EBV-TR increases survival ratios through a mechanism other than the increased expression level of dhfr gene, i.e. the mechanism may involve increased integration of the vector.

Detailed Description Text (11):
Drug selection of transfected cells with a vector containing EBV-TR

Detailed Description Text (12):
CHO (dhfr-) cells were separately transfected with 5 .mu.g of pSH134 and 5 .mu.g of pSH135 using cationic liposome DMRIE-C reagent according the protocol provide by Life Technology. Transfected cells (5.times.10.sup.5 cells per 96 well plate) were selected in serum-free medium supplemented with r-insulin, transferrin and 50 nM MTX lacking hyphoxanthine and thymidine. Growth-positive wells were counted at 2-weeks after intitial selection in selection medium with 50 nM MTX. No MTX-resistant clones were derived from mock transfected cells. Results are shown in Table 1. Exp. 1 (IL-4dm) was performed in the serum-free medium lacking hypoxanthine and thymidine supplemented with 50 nM methotrexate. Exp.2 (IL-4dm) was performed in serum (5%) containing selection medium supplemented with 50 nM methotrexate. Exp.3 (BDD-FVIII) and Exp. 4 (BDD-FVIII) were performed in the serum-free selection medium as in Exp. 1.

Detailed Description Text (13):
Two weeks after seeding the cells in selection medium, pSH135 (with EBV-TR) transfected cells showed an approximately 10 fold higher selection ratio than those transfected with pSH134 (without EBV-TR), although EBV-TR showed no enhancing function on IL-4 expression (FIG. 3). CHO (dhfr-) cells were also transfected with 5 .mu.g of pCIS25D and 5 .mu.g of pCIS25DTR using DMRIE-C reagent. Cells were selected under the same conditions as described above. Cells transfected with pCIS25DTR showed about 3-fold to about 16-fold higher selection ratio than those transfected with pCIS25D (Table 1). These results indicate that this sequence of EBV-TR in the expression vector can be used for increased integration of the vector in vivo for gene therapy.

Detailed Description Text (15):
Selection for high producing cell lines under serum-free conditions

Detailed Description Text (16):
CHO (dhfr-negative) cells transfected with pSH135 were plated onto 96-well plates (5.times.10.sup.5 cells per plate) using a serum-free selection medium supplemented with transferrin, recombinant insulin, and methotrexate (50 nM). The selection

medium lacks hypoxanthine and thymidine. After three months of amplification (50 and 100 nM MTX), one of the intitial populations, denoted 1G9, was adapted to suspension culture using a shake flask. The high level of IL-4dm productivity (.about.5 pg/c/d) was observed to continue for at least about 10 weeks in a serum-free and albumin-free medium supplemented with transferrin and recombinant insulin.

Detailed Description Text (19):
One of the CHO clones secreting BDD-FVIII, which was derived from pCIS25DTR transfected CHO cells as described in Example 2, was tested for its production stability under the absence of the selection drug (MTX). This clone continued to secrete BDD-FVIII during a six month period of growth in a medium lacking MTX. All single cell clones derived from this clone were also secretion positive. These results indicate that the integration of the vectors containing an EBV-TR sequence is a stable integration.

Detailed Description Text (21):
The derivation of stable cell lines which secrete high levels of proteins is a very tedious and labor-intensive task. This is at least partially due to the low chance of stable integration and amplification of the gene of interest. Large numbers of drug resistant clones generally need to be screened to obtain high secreting clones. Therefore, we have described here that vectors having an EBV-TR sequence result in an enhanced drug selection ratio, indicating a high integration ratio of transferred genes. As shown in Table 1, it was possible to select and to amplify transfected cells even under serum-free conditions.

Detailed Description Paragraph Table (1):
TABLE 1 Drug-selection ratios from the transfected CHO (dhfr-) cells using the IL- 4 dm and BDD-FVIII expression vectors linked with and without EBV-TR. Growth-positive wells/total wells (1) Vector w/o EBV-TR.sup.1 (2) Vector with EBV-TR.sup.2 % growth of (2) over (growth.sup.+ /total) (growth.sup.+ /total) % growth of (1).sup.3 Exp. 1 (IL-4 dm) 28/960 (2.9%) 238/960 (24.8%).sup.4 8.5 Exp. 2 (IL-4 dm) 51/576 (8.8%) 520/864 (60.1%).sup.4 6.8 Exp. 3 (BDD-FVIII) 48/1344 (3.6%) 2227/3840 (58%).sup.4 16 Exp. 4 (BDD-FVIII) 64/864 (7.4%) 288/1056 (27%).sup.4 3.6 .sup.1 Expression vectors lacking EBV-TR were used for transfection. .sup.2 Expression vectors containing EBV-TR were used for transfection. .sup.3 This ratio indicates growth.sup.+ ratios of cells transfected with the vector having EBV-TR over cells transfected with the vector lacking EBV-TR. .sup.4 Actual number of growth-positive colonies are much higher than growth-positive numbers counted from each well, because multiple colonies were growing in many growth-positive wells.

CLAIMS:

3. A method of introducing an expression vector into mammalian cells in vitro comprising the steps of:

a) contacting the mammalian cells with the expression vector under conditions which allow uptake of the expression vector by the cells, the expression vector comprising a first DNA sequence coding for a heterologous protein, a second DNA sequence encoding an amplifiable marker, and an EBV-TR sequence (SEQ ID NO:1);

b) growing cells obtained from step a) in a selection medium under conditions which allow selection for resistant cells; and

c) recovering cells obtained from step b) which express the heterologous protein.

4. The method of claim 3 further comprising the step of:

d) growing cells recovered in step c) under conditions which allow further selection to occur.

9. A process for obtaining a CHO cell line which expresses IL-4dm, the process comprising the steps of

a) obtaining CHO cells,

b) contacting said CHO cells with a vector comprising an EBV-TR sequence (SEQ ID NO:1) and a coding sequence for IL-4dm under conditions which allow for the uptake of the vector by the cells,

c) establishing a CHO cell line which expresses IL-4dm from the result of step b).

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DOCUMENT-IDENTIFIER: US 6136599 A
TITLE: Human hybrid host cell for mammalian gene expression

Abstract Text (1):
Human/human hybrid cells were made via fusion of human embryonic kidney cells (293S) and modified Burkitt's lymphoma cells (2B8). The fusion cells are useful as host cells for the recombinant expression of mammalian genes. The advantages of using these hybrid clones of human kidney- and B-cells, called HKBs, for mammalian gene expression, include (i) the cells are negative for immunoglobulin expression, (ii) the cells grow easily in plasma protein-free medium (with or without the addition of recombinant insulin) as suspension cultures in a shake flask or in a fermenter (iii) the cells are very susceptible for transfection of DNA, and (iv) the cells secrete high levels of heterologous recombinant proteins, such as recombinant monoclonal antibodies, soluble ICAM-1, rIL-4, and rFVIII.

Brief Summary Text (3):
This invention relates generally to the genetically engineered mammalian cell lines for the production of biologically active protein. Specifically, the invention is concerned with human hybrid cell clones derived from the fusion process of human embryonic kidney (293S) cells and Burkitt's lymphoma cells. These human hybrid cells can be used for the production of heterologous proteins.

Brief Summary Text (5):
To date, most therapeutic recombinant proteins have been produced from non-human mammalian cells. Some examples are:

Brief Summary Text (6):
Chinese hamster ovary (CHO) (dhfr-) cells (Urlaub et al., 1980, Proc Natl Acad Sci U.S.A. 77: 4216-4220) with the amplifiable selection marker dihydrofolate reductase (Kaufman et al., 1982, Mol. Biol. 159: 601-621; Gasser et al., 1982, Proc Natl Acad Sci U.S.A. 79: 6522-6526) have been used for the production of therapeutic recombinant protein.

Brief Summary Text (7):
A variety of recombinant therapeutic proteins are known to be produced in mammalian cells, e.g. recombinant factor VIII (rFVIII) (Kaufman et al., 1988, J Biol Chem 263: 6352-6362), tissue plasminogen activator (tPA) (U.S. Pat. No. 4,766,075 to Goeddel et al., 1988), erythropoietin (EPO) (U.S. Pat. No. 4,703,008 to Lin, 1987), and monoclonal antibodies (mAbs) (U.S. Pat. No. 4,816,397 to Boss et al., 1989).

Brief Summary Text (8):
Baby hamster kidney (BHK) cells (BHK21) were used for production of rFVIII after G418 selection and methotrexate (MTX) amplification of G418 resistant cells (U.S. Pat. No. 4,965,199 to Capon et al., 1990).

Brief Summary Text (9):
Mouse myeloma (NS0) cells were used for production of engineered human anti-TNF antibody (EHAT). (U.S. Pat. No. 4,816,397 to Boss et al., 1989). However, this cell line produces proteins having mouse specific carbohydrate patterns which are not favored for human use.

Brief Summary Text (10):
A human cell line, Namalwa (of Burkitt's lymphoma origin), was used for production of alpha-interferon by Wellcome Research Laboratory and for the production of pro-urokinase (Satoh et al., 1996, Cytotechnology 18: 167-185, 1996), tissue-plasminogen activator (t-PA) (Khan et al., 1995, Biochem Soc Trans 23: S99), granulocyte-macrophage colony-stimulating factor (Okamoto et al., 1991, Arch Biochem Biophys 286: 562-568), interferons and lymphotoxin (Hosoi et al., 1991, Cytotechnology 5: 17-34), and granulocyte colony stimulating factor (Hosoi et al., 1991, Cytotechnology 7: 25-32) by Tokyo Research Laboratories. However, these cells were very difficult to transfect with DNA.

Brief Summary Text (11):
Walls et al. (1989, Gene 81: 139-149) have reported on the use of the dhfr/MTX co-amplification strategy to express functional protein C in human embryonic kidney cells (293S cells). 293 cells (Stillman et al., 1985, Mol. Cell. Biol. 5: 2051-2060) are known as making large aggregates in suspension, especially under high calcium concentration (>100 .mu.M), which promotes larger aggregation and lower cell viability (Peshwa et al., 1993, Biotech and Bioeng 41: 179-187). All references cited are herein incorporated by reference.

Brief Summary Text (13):
Clones of hybridized human cells have now been established which are easily transfected by electroporation or cationic liposome and which are easily adapted to growth in suspension culture. Heterologous proteins can be expressed using low levels (50-100 nM) of MTX amplification in a human cell environment. In addition, the cells are easily adapted to growth in serum-free medium.

Brief Summary Text (14):
These cells are the product of a fusion between human embryonic kidney (293S) cells and Burkitt's lymphoma cells. See FIG. 1 for the summary. These hybrid clones, called HKBs, harbor a defective EBV-genome derived from HH514-16, which is a cell line originating from Burkitt's lymphoma cells, P3HR1 (Hinuma et al., 1967, J Virol 1: 1045-1051). P3HR1 cells harbor non-immortalizing EBV. HH514-16 is a clone of P3HR1 (Hinuma et al., 1967, J Virol 1: 1045-1051) which harbors non-immortalizing EBV. HH514-16 has lost a het-DNA, a latency-interrupting DNA, during the cloning process (Rabson et al., 1983, Proc Natl Acad Sci U.S.A. 87: 3660-3664). Therefore, EBV of HKB clones is non-immortalizing virus and stays as a latency.

Brief Summary Text (15):
HKBs are human hybrid host cells suitable for the recombinant production of therapeutic proteins. These host cells are obtained by the hybridization of different parent cell lines, each having different advantageous characteristics. Host cells which possess advantageous characteristics of each of the parent cell lines are obtained from the cells resulting from the hybridization.

Brief Summary Text (16):
The host cells may be genetically engineered to express high levels of a wide range of proteins. Proteins which may be produced by the engineered host cells include, but are not limited to, soluble ICAM-1, recombinant interleukin-4 (IL-4), rFVIII, BDD-FVIII B domain deleted factor VII, (as disclosed in the related application to Cho et al. Ser. No. 09/209,916, incorporated herein by reference), tPA, and EPO, and derivatives of these proteins. It has also been found that, although endogenous immunoglobulin (Ig) was not expressed, recombinant Ig expression from the engineered host cells was was high. Proteins produced from HKB11 cells have human specific glycosylation profile. Therefore, the clones are optimal host cells for the production of gene-engineered Ig and other proteins.

Brief Summary Text (17):
As used herein, a cell of Burkitt's lymphoma origin is a cell that is a Burkitt's lymphoma cell, derived from a Burkitt's lymphoma cell, derived from another cell of Burkitt's lymphoma origin, or a cell resulting from mitotic division of any of the above. "Derived from" in this context is intended to include, but not be limited to, normal mitotic cell division and processes such as transfections, cell fusions, or other genetic engineering or cell biology techniques used to alter cells or produce cells with new properties. Similarly, a cell of human embryonic kidney origin is a

cell that is a human embryonic kidney cell, derived from a human embryonic kidney cell, derived from another cell of human embryonic kidney origin, or a cell resulting from the mitotic division of any of the above. Also, a cell of 293S origin is a cell that is a 293S cell, derived from a 293S cell, derived from another cell of 293S origin, or a cell resulting from the mitotic division of any of the above. A heterologous protein is a protein that a cell has been engineered to produce.

Drawing Description Text (2):
FIG. 1 shows a summary of the derivation of the HKB cells.

Drawing Description Text (3):
FIG. 2 shows physical maps of expression vectors mentioned in the text. All plasmids are constructed based on a pBR322 backbone and contain a dhfr expression unit. All genes coding proteins of interest are under the regulation of CMV enhancer/promoter (CMVe/p); 5'intron (MIS or CIS) was positioned at the 5'end of the genes, except BZLF1. Poly A signal region was indicated as pA. Both plasmids, pSH157 and pCIS25DTR, contain a sequence of EBV-TR (402 bp).

Drawing Description Text (4):
FIG. 3 shows a comparison of host cell lines for gene expression in transient transfection assay. Transfections were performed under the same conditions: the same number of cells of 293S, 2B8, and HKB11 were transfected with the same amount of plasmid DNAs using the same transfection agent. Tissue culture fluids were harvested at 2 days after transfection. Protein production levels were determined by ELISA (IgG and ICAM-1; measured as ng protein/10.sup.6 cells/2 days) and by Coatest.RTM. assay kit (rFVIII; measured as milliunits/10.sup.7 cells/2 days).

Detailed Description Text (3):
HH514-16 was kindly provided by Dr. George Miller (Yale University). 293S cells were obtained from Dr. Brad Zerler (Molecular Therapeutic Institute, West Haven, Conn.) 293S cells are 293 cells (ATCC CRL-1573) which have been adapted to grow in suspension culture (Stillman et al., 1985, Mol Cell Biol 5: 2051-2060).

Detailed Description Text (5):
All expression vectors used in this report were basically pBR322-based plasmid with function dhfr gene expression segement. Physical maps of expression vectors are described in FIG. 2. Plasmids, pSH157 and pCIS25DTR, have also terminal repeat sequence of Epstein-Barr virus (EBV-TR). See patent application to Cho and Chan designated MSB-7254, "Terminal repeat sequence of Epstein-Barr virus enhances drug selection ratio," for the EBV-TR sequence. The vector pSH13 1, which has been deposited with the American Type Culture Collection, ATCC 98879, may be used to generate expression vectors for a chosen protein as described in Cho and Chan (MSB-7254, supra.)

Detailed Description Text (13):
Derivation of HAT-Sensitive and G418-Resistant Burkitt's Lymphoma Cell Line

Detailed Description Text (14):
To obtain HAT-sensitive cells, hypoxanthine-guanine phosphoribosyl transferase (HGPRT)-deficient cell lines (Szybalska et al., Proc. Natl. Acad. Sci. U.S.A. 48: 2026-2034, 1962; Littlefield, Proc. Natl. Acad. Sci. U.S.A. 50: 568-576, 1963) were established by the standard protocol described by Siadak et al. (U.S. Pat. No. 4,834,975, 1989).

Detailed Description Text (15):
HH514-16 cells (obtained from Dr. G. Miller, Yale University), which are free of EBV het-DNA (Rabson et al., 1983, Proc Natl Acad Sci U.S.A. 87: 3660-3664), were treated with 300 .mu.g/ml of methanesulfonic acid ethyl ester (MSE) (Sigma, St. Louis, Mo.) in RPMI-1640 suspension medium (Life Science, Gaithersburg, Md.) supplemented with 15% fetal bovine serum (FBS) (Hyclone, Logan, Utah) for 24 hours. After washing the cells with medium, cells were plated in medium containing 6-thioguanine (6TG) (Sigma) (5 .mu.g/ml) to select for HGPRT-negative cells. The concentration of 6TG was increased from 5 .mu.g/ml to 30 .mu.g/ml during the six month selection period. The cells were then tested for their sensitivity to HAT-containing medium. Single cell clones (SCCs) were obtained from limiting dilution cloning (one cell per well

in 96-well plates) of HAT-sensitive population A5. One of the SCCs, A5/ID7, was transfected with pSV2neo, which has neo gene under SV40 promoter in pBR vector, to obtain G418-resistant cells. One of the G418 (1.5 mg/ml) resistant SCCs, referred to as 2B8 (deposited with the American Type Culture Collection, 10801 University Blvd., Manassas, Va. 20110, on Sep. 16, 1999 and granted accession No. CRL-12569), was used for fusion.

Detailed Description Text (17):
Cell Fusion and Derivation of Single Cell Clones

Detailed Description Text (18):
Cell fusion was primarily performed according to the polyethylene glycol (PEG) fusion method described by Kennett (1979, Meth Enzymol 58: 5-359). Five million each of 293S and 2B8 cells in logarithmic growth were washed with PBS without Ca.sup.++ and Mg.sup.++ (Life Technologies, Rockville, Md.) and seeded onto one well of a 6-well plate pre-treated with peanut agglutinin (Sigma) (5 .mu.g/ml). The 6-well plates loaded with cells were centrifuged at 400 g for 6 minutes in a Beckman J-6M/E centrifuge (Beckman, Palo Alto, Calif.). After removing the PBS from the well, cells were treated with 2 ml of 40% (w/v) PEG (Sigma) for one minute. As a control, one well was not treated with PEG. Cells were washed three times with 5 ml PBS containing 5% DMSO followed by three PBS washes. Cells were incubated with fresh medium supplemented with 15% FBS for 25 minutes. Cells were seeded onto 96-well plates (1.2.times.10.sup.6 cells per plate) using the fresh medium containing G418 (1 mg/ml) and HAT (Life Technology) supplemented with 15% FBS. The cells were fed twice a week using the selection medium. In this example, for the initial selection, the 293S cells had the desirable characteristic of lacking sensitivity to the HAT containing medium, and, similarly, the 2B8 cells had the desirable characteristic of being resistant to G418.

Detailed Description Text (19):
While the fused cells grew under selective conditions, the mixed cells did not grow under the same conditions. Three weeks after selection, the initial populations were transferred to larger formats. To obtain SCCs, the stably growing twenty initial populations were mixed and subjected to limiting dilution cloning (one cell per well) using the selective medium. Nineteen SCCs were selected from 15.times.96-well plates after careful monitoring the individual clones using a microscope. The SCCs derived from the fusion experiment were referred to as HKB cells (Hybrid cells of human kidney- and B-cells). Seven SCCs were chosen from the transfection study. These seven SCCs were further tested for the stable production of various proteins. One of the seven SCCs, HKB11, (deposited on Sep. 16, 1998 with American Type Culture Collection, 10801 University Blvd., Manassas, Va. 20110, and granted accession number CRL-12568) was chosen as a preferred mammalian cell host for the production of heterologous proteins. See FIG. 1 for the summary.

Detailed Description Text (22):
Although all hybrid cells were selected under selective conditions, the hybrid status was confirmed by counting the chromosome numbers in cells. Indeed, all of the HKBs showed a modal chromosome number of 90-110. These numbers were similar with the sum of modal chromosome numbers of 293S and 2B8 (64 and 47, respectively, according to ATCC data). 293S (Stillman et al., 1985, Mol Cell Biol 5: 2051-2060) is suspension adapted 293 (ATCC CRL-1573).

Detailed Description Text (23):
All hybrid cell clones were tested for endogenous Ig (mu and kappa) expression by direct immunofluorescence assay using methanol fixed cells to determine which cell clones secrete endogenous Ig. In repeated experiments over a longer period, these cells were observed to be negative for mu- and kappa-chains expression based on immunofluorescence test (Table 1) and ELISA (data not shown).

Detailed Description Text (24):
Table 1 shows the results observed from the immunofluorescence testing of three types of cells. Cells were resuspended in PBS and applied as a smear on a glass slide. After drying the cells, the slides were fixed in a cold (-20.degree. C.) methanol for 5 minutes. The cells were stained with FITC-anti human kappa and anti human mu chains (1:20 dilution) (Zymed Laboratories, Inc., So. San Francisco,

Calif.) in a humidified chamber at 37.degree. C. for 45 minutes. After rinsing slides with PBS for 10 minutes, slides were mounted with cover glass using PBS/Glycerol (1:1). Cells were observed in a fluorescence microscope (Carl Zeiss, Inc., Thornwood, N.Y.).

Detailed Description Text (25):
The human specific pattern of sialic acid linkage, alpha(2-6) sialyltransferase was confirmed by a FACS analysis of the HKB cells using FITC conjugated Sambucus nigra lectin (SNA) (Sigma, St. Louis, Mo.) Protein secreted from the HKB cells (clone 1G2) showed alpha(2-3) and alpha(2-6) linkage of sialic acid of glycosylation profile analyzed by oligosaccharide fingerprinting method (data not shown). This observation indicates that HKB cells derived from fusion of 293S and 2B8 cells maintained human specific glycosylation enzymes.

Detailed Description Text (26):
To test if these hybrid cells have the capacity to express foreign genes, the cells were transfected with expression vectors for ICAM-1 and IgG (anti-TNF antibody). To test secretion of IgG, HKB cells (5.times.10.sup.6 cells) were transfected by electroporation with 10 .mu.g of plasmid DNA providing for functional expression of heavy (gamma) and light (kappa) chains. To test secretion levels of soluble ICAM-1, HKB cells (5.times.10.sup.6 cells) were transfected by electroporation with 10 .mu.g of plasmid DNA providing for functional expression of soluble ICAM-1. Secretion levels were determined by an ELISA for IgG or ICAM-1. All 19 SCCs secreted relatively high levels of ICAM-1 (100-500 ng/ml/2d) and IgG (30-200 ng/ml/2d) in a transient transfection assay (Table 2). These data indicate that the hybrid cells support the expression of transfected Ig genes, although endogenous Ig gene expression was extinguished.

Detailed Description Text (27):
Epstein Barr virus (EBV) exists as episomes in 2B8 cells. All the SCCS were positive for EBNA-1 expression (data not shown), which indicates that they are positive for EBV. However, it was not known whether a complete EBV genome still existed in the hybrid cells. Therefore, the status of the EBV genome in the hybrid cells was tested by transfecting the cells with the EBV genome fragment BZLF1, a latency interrupting trans-activating gene. HKB cells (5.times.10.sup.6 cells) were transfected with 10 .mu.G of plasmid DNA (pSH121) allowing functional expression of BZLF1. Detection of EBV capsid antigen (EBV-VCA) was performed by indirect immunofluorescence using human serum containing anti EBV-VCA titer and FITC conjugated anti-human IgG. Technical details of the immunofluorescence test is described above. As shown in Table 3, mock transfected cells were negative for expression of EBV capsid antigen (EBV-VCA), which indicated that they were negative for EBV replication. However, a small percentage of the transfected cells were positive for the antigen expression. These data indicate that the hybrid cells harbor EBV genome in its latent form.

Detailed Description Text (28):
The hybrid cells were adapted to serum-free medium by serial two fold reduction of FBS in shake flasks. After two weeks, cells were growing in the medium without FBS. The cells grew as small aggregates in shake flasks. In contrast to 293S cells, the hybrid cells were easily adaptable to serum-free suspension cultures. Hybrid cells in serum-free and albumin-free medium supplemented with transferrin and insulin could be maintained for more than one year in suspension culture using shake flasks.

Detailed Description Text (29):
To compare secretion levels of transfected gene products, one of the clones, HKB11, and parental cells, 293S and 2B8, were transfected with pSM98 (soluble ICAM-1), pSH125 (anti-TNF IgG), and pCISF8 (rFVIII). As shown in FIG. 3, secretion levels of ICAM-1 and IgG were much higher (approximately 10-fold) in HKB11 cells than 293S cells. Secretion levels of both proteins from 2B8 were undetectable. Secretion levels of rFVIII in HKB11 cells was similar to that of 293S cells. These data indicate that transfection efficiency of HKB11 cells are much better than parental cells.

Detailed Description Text (32):
Hybrid clones were tested for the stable expression of proteins in a gene

amplifiable system (dhfr/MTX). Cells were first transfected with an appropriate expression vector, and then the transfected cells (usually 10.sup.6 cells per 96-well plate) were seeded and selected/amplified with the selection medium lacking hypoxanthine and thymidine, but supplemented with FBS and MTX (50 nM). After the first screening of the plates, the cells from the high secreting wells were chosen and transferred to the 6-well plates. The cells in the 6-well plates were further amplified with increasing concentrations of MTX (100, 200, and 400 nM) in medium. The initial populations of the 6-well plates were further screened for deriving the best population. Eventually, these populations were used for cloning of single cell derived clones. As shown in table 4, HKB11 cells were optimal for producing high levels of heterologous human proteins. For the production of ICAM-1, Ig, and an IL-4 derivative, HKB11 cells were as good as CHO cells (data are not shown). However, HKB clones grew faster than CHO clones and could be adapted easily to suspension culture and to serum-free conditions. In case of BDD-FVIII production, HKB11 clones showed about tenfold greater productivity than CHO clones. The above results indicate that HKB11 cells are an optimal human cell host useful for the expression of human therapeutic proteins.

Detailed Description Text (33):
The cell lines derived by the above process included (i) soluble ICAM-1 secreting clones (10 pg/cell/day) derived from HKB11 cells transfected with pSM98 after amplification in 100 nM MTX, (ii) monoclonal antibody (anti-TNF) secreting single cell clones (12 pg/cell/day) derived from the

Detailed Description Text (34):
HKB13 transfected with pSS125 after amplification in 50 nM MTX and limiting dilution cloning without MTX, (iii) truncated rFVIII (BDD-FVIII) secreting single cell clones (5-10 .mu.U/c/d, in serum-free condition) derived from HKB11 cells transfected with pCIS25DTR after amplification (400 nM MTX) and limiting dilution cloning without MTX, and (iv) IL-4 derivative (IL-4 selective agonist, IL-4SA; mutated two positions of amino acid, T13D and R121E) secreting clones (5 pg/c/d) derived from HKB11 transfected with pSH157 after MTX amplification. The IL-4SA secreting HKB clone, 1G2, (deposited on May 19, 1999 with American Type Culture Collection, 10801 University Blvd., Manassas, Va. 20110, and granted accession number PTA-87) was used for relatively quick production of small amounts of protein (gram quantity). See FIG. 2 for the physical map of the expression vectors mentioned above.

Detailed Description Text (36):
The hybrid human cell lines described herein display desirable characteristics possessed by their parent cell lines. HKB cell lines, which are produced by the fusion of human embryonic kidney cells (or cells derived from human embryonic kidney cells) with Burkitt's lymphoma cells (or cells derived from Burkitt's lymphoma cells), are useful for developing cell lines for the expression of heterologous proteins.

Detailed Description Text (37):
The initial purpose of establishing the hybrid cell lines of 293S and 2B8 was to resolve the aggregation problem of 293S cells, which tend to clump when grown in suspension culture (an undesirable characteristic). The HKB cells that were developed from the hybridization process grow as a monolayer when cultured in T-flasks. However, these cells grow as suspension cells (do not form large aggregates) when they are cultured in suspension mode. HKB cells are very easy to handle for transfection, and transfection efficiency is much higher than with 293S cells.

Detailed Description Text (38):
Although the transformation or hybridization event required in most cases to produce a stable cell line may result in altered glycosylation profiles (Yamashita, 1989, J Biol Chem 264: 2415-2423), it was found that HKB11, which is a somatic hybrid cell line, has typical human glycosylation enzymes, e.g. alpha(2-3) and alpha(2-6) sialyl transferases. Moreover, proteins produced from transfected HKB cells have normal human glycosylation patterns of alpha(2-3) and alpha(2-6) sialic acid linkages.

Detailed Description Text (39):
In summary, HKBs are hybrid human cells having desirable characteristics from each

of the parental cell lines; namely, growing easily in suspension culture without aggregation (as observed with 2B8 cells) and ease of transfection and desirable secretion characteristics (as observed with 293S cells). The preferred hybrid human cell line, HKB11, is negative for immunoglobulin gene expression as are 293S cells. This trait is advantageous in cases where it is desired to recombinantly produce monoclonal antibodies from human cells. The discovery that fusion of human cells has resulted in cells having advantageous characteristics serves to encourages further fusion studies using 293S and other cell lines of B-cell origin, e.g. Namalwa and 6F11, to obtain new combinations of traits in the hybrid cells from fusing different parental cell lines.

Detailed Description Paragraph Table (1):
TABLE 1 _____ Detection of Ig-gene expression. Gene expression Cells Heavy chian (mu) Light chain (kappa)
_____ 293S negative negative 2B8 positive positive HKB negative negative _____

Detailed Description Paragraph Table (4):
TABLE 4 _____ Heterologous protein production from HKB clones. Protein Cell host MTX amplification Specific Productivity
_____ ICAM-1 HKB11 100 nM 10 pg/c/d.sup.1) Ig HKB13 50 nM 12 pg/c/d BDD-FVIII HKB11 400 nM 5-10 uU/c/d.sup.2) IL-4SA HKB11 100 nM 5 pg/c/d.sup.3) _____ .sup.1) ICAM-1 production level from HKB clones were similar with that from 293S cells. HKB clone secreting ICAM1 was easily adaptable to suspension culture, while 293S clones were very difficult to adapt to suspension culture. .sup.2) The secretion level of BDDFVIII secretion was about 10 times higher than those from the clones derived from CHO cells transfected with the same expression vector. .sup.3) Gram quantity of IL4SA(T13D/R121E) production was possible 6 months post transfection using HKB11, while it took a few months longer using CHO cells in simultaneous experiments using the same expression vector and similar process.

Other Reference Publication (1):
Stillman, et al. (Molec. & Cell. Biol., 1985; 2051-2060).

Other Reference Publication (4):
ATCC Cell Lines and Hybridomas (8th Ed., 1994; 149).

CLAIMS:

1. A cell derived from the fusion of a cell of human embryonic kidney origin with a 2B8 cell (ATCC deposit number CRL-12569).

2. A cell according to claim 1 wherein the cell of human embryonic kidney origin is a 293 cell.

3. A cell according to claim 1 wherein the cell of human embryonic kidney origin is a 293S cell.

4. A cell according to claim 1 which expresses a heterologous protein.

5. A cell according to claim 4, wherein the heterologous protein is selected from the group consisting of FVIII, BDD-FVIII, monoclonal antibody, anti-TNF antibody, rIL4, tPA, and EPO.

6. A cell line designated HKB11 (ATCC deposit number: CRL-12568).

7. An HKB11cell line which has been engineered to express a heterologous protein.

8. A cell line according to claim 7 wherein the protein is ICAM-1.

9. A cell line according to claim 7 wherein the protein is BDD-FVIII.

10. A cell line according to claim 7 wherein the protein is a monoclonal antibody.

11. A cell line according to claim 10 wherein the monoclonal antibody is anti-TNF.

12. A cell line according to claim 7 wherein the protein is rIL4.

13. A cell line according to claim 7 wherein the protein is FVIII.

14. A cell line according to claim 7 wherein the protein is tPA.

15. A cell line according to claim 7 wherein the protein is EPO.

16. A cell line according to claim 7 wherein the protein is IL-4SA (T13D/R121E).

17. A cell line according to claim 7 wherein the protein is a human protein having a human glycosylation profile.

18. A method of producing hybrid human cells useful for expression of a heterologous protein comprising the steps of

a) obtaining cells of human embryonic kidney origin,

b) obtaining 2B8 cells (ATCC deposit number CRTL-12569),

c) contacting the cells of step a) with the cells of step b) under conditions which allow cell fusion to occur,

d) screening the cells resulting from step c) for cells which are useful for the expression of a heterologous protein.

19. The method of claim 18 wherein the cells of human embryonic kidney origin are 293 cells.

20. The method of claim 18 wherein the cells of human embryonic kidney origin are 293S cells.

21. The cell line according to claim 16 designated 1G2 (ATCC deposit number PTA-87).

22. The method of claim 18 wherein

i) the cells of step a) have a first desirable characteristic,

ii) the cells of step b) have a second desirable characteristic, and

iii) screening the cells in step d) provides cells which display at least one desirable characteristic of each of the cells of steps a) and b).

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NEWS	3	SEP 09	CA/CAplus records now contain indexing from 1907 to the present
NEWS	4	AUG 05	New pricing for EUROPATFULL and PCTFULL effective August 1, 2003
NEWS	5	AUG 13	Field Availability (/FA) field enhanced in BEILSTEIN
NEWS	6	AUG 18	Data available for download as a PDF in RDISCLOSURE
NEWS	7	AUG 18	Simultaneous left and right truncation added to PASCAL
NEWS	8	AUG 18	FROSTI and KOSMET enhanced with Simultaneous Left and Righ Truncation
NEWS	9	AUG 18	Simultaneous left and right truncation added to ANABSTR
NEWS	10	SEP 22	DIPPR file reloaded
NEWS	11	DEC 08	INPADOC: Legal Status data reloaded
NEWS	12	SEP 29	DISSABS now available on STN
NEWS	13	OCT 10	PCTFULL: Two new display fields added
NEWS	14	OCT 21	BIOSIS file reloaded and enhanced
NEWS	15	OCT 28	BIOSIS file segment of TOXCENTER reloaded and enhanced
NEWS	16	NOV 24	MSDS-CCOHS file reloaded
NEWS	17	DEC 08	CABA reloaded with left truncation
NEWS	18	DEC 08	IMS file names changed

NEWS EXPRESS	NOVEMBER 14 CURRENT WINDOWS VERSION IS V6.01c, CURRENT MACINTOSH VERSION IS V6.0b(ENG) AND V6.0Jb(JP), AND CURRENT DISCOVER FILE IS DATED 23 SEPTEMBER 2003
NEWS HOURS	STN Operating Hours Plus Help Desk Availability
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NEWS PHONE	Direct Dial and Telecommunication Network Access to STN
NEWS WWW	CAS World Wide Web Site (general information)

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FILE 'HOME' ENTERED AT 15:56:43 ON 08 DEC 2003

=> file medline, uspatful,dgene, embase, fsta, wpids, jicst, biosis

COST IN U.S. DOLLARS	SINCE FILE ENTRY	TOTAL SESSION
FULL ESTIMATED COST	0.21	0.21

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=> s cell production
   6 FILES SEARCHED...
L1       12334 CELL PRODUCTION

=> s cell production () method
   6 FILES SEARCHED...
L2         184 CELL PRODUCTION (W) METHOD

=> s l2 and l1
L3         184 L2 AND L1

=> s l3 and human
L4          14 L3 AND HUMAN

=> s l1 and human
   4 FILES SEARCHED...
L5        6547 L1 AND HUMAN

=> s l3 and vector
L6          20 L3 AND VECTOR

=> s l5 and vector
L7        1453 L5 AND VECTOR

=> s l6 and promoter
L8           4 L6 AND PROMOTER

=> s l7 and promoter
L9        1016 L7 AND PROMOTER

=> d l8 ti abs ibib tot

L8   ANSWER 1 OF 4  USPATFULL on STN
TI     Compositions and methods for the therapy and diagnosis of colon cancer
AB     Compositions and methods for the therapy and diagnosis of cancer,
       particularly colon cancer, are disclosed. Illustrative compositions
       comprise one or more colon tumor polypeptides, immunogenic portions
       thereof, polynucleotides that encode such polypeptides, antigen
       presenting cell that expresses such polypeptides, and T cells that are
       specific for cells expressing such polypeptides. The disclosed
```

compositions are useful, for example, in the diagnosis, prevention and/or treatment of diseases, particularly colon cancer.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

ACCESSION NUMBER: 2003:237907 USPATFULL
TITLE: Compositions and methods for the therapy and diagnosis of colon cancer
INVENTOR(S): King, Gordon E., Shoreline, WA, UNITED STATES
Meagher, Madeleine Joy, Seattle, WA, UNITED STATES
Xu, Jiangchun, Bellevue, WA, UNITED STATES
Secrist, Heather, Seattle, WA, UNITED STATES
Jiang, Yuqiu, Kent, WA, UNITED STATES
PATENT ASSIGNEE(S): Corixa Corporation, Seattle, WA, UNITED STATES, 98104 (U.S. corporation)

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 2003166064	A1	20030904
APPLICATION INFO.:	US 2002-99926	A1	20020314 (10)

RELATED APPLN. INFO.: Continuation-in-part of Ser. No. US 2001-33528, filed on 26 Dec 2001, PENDING Continuation-in-part of Ser. No. US 2001-920300, filed on 31 Jul 2001, PENDING

	NUMBER	DATE
PRIORITY INFORMATION:	US 2001-302051P	20010629 (60)
	US 2001-279763P	20010328 (60)
	US 2000-223283P	20000803 (60)

DOCUMENT TYPE: Utility
FILE SEGMENT: APPLICATION
LEGAL REPRESENTATIVE: SEED INTELLECTUAL PROPERTY LAW GROUP PLLC, 701 FIFTH AVE, SUITE 6300, SEATTLE, WA, 98104-7092
NUMBER OF CLAIMS: 17
EXEMPLARY CLAIM: 1
LINE COUNT: 8531
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L8 ANSWER 2 OF 4 USPATFULL on STN
TI Compositions and methods for the therapy and diagnosis of pancreatic cancer
AB Compositions and methods for the therapy and diagnosis of cancer, particularly pancreatic cancer, are disclosed. Illustrative compositions comprise one or more pancreatic tumor polypeptides, immunogenic portions thereof, polynucleotides that encode such polypeptides, antigen presenting cell that expresses such polypeptides, and T cells that are specific for cells expressing such polypeptides. The disclosed compositions are useful, for example, in the diagnosis, prevention and/or treatment of diseases, particularly pancreatic cancer.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

ACCESSION NUMBER: 2003:106233 USPATFULL
TITLE: Compositions and methods for the therapy and diagnosis of pancreatic cancer
INVENTOR(S): Benson, Darin R., Seattle, WA, UNITED STATES
Kalos, Michael D., Seattle, WA, UNITED STATES
Lodes, Michael J., Seattle, WA, UNITED STATES
Persing, David H., Redmond, WA, UNITED STATES
Hepler, William T., Seattle, WA, UNITED STATES
Jiang, Yuqiu, Kent, WA, UNITED STATES
PATENT ASSIGNEE(S): Corixa Corporation, Seattle, WA, UNITED STATES, 98104 (U.S. corporation)

	NUMBER	KIND	DATE

PATENT INFORMATION:	US 2003073144	A1	20030417
APPLICATION INFO.:	US 2002-60036	A1	20020130 (10)

	NUMBER	DATE
PRIORITY INFORMATION:	US 2001-333626P	20011127 (60)
	US 2001-305484P	20010712 (60)
	US 2001-265305P	20010130 (60)
	US 2001-267568P	20010209 (60)
	US 2001-313999P	20010820 (60)
	US 2001-291631P	20010516 (60)
	US 2001-287112P	20010428 (60)
	US 2001-278651P	20010321 (60)
	US 2001-265682P	20010131 (60)

DOCUMENT TYPE: Utility
FILE SEGMENT: APPLICATION
LEGAL REPRESENTATIVE: SEED INTELLECTUAL PROPERTY LAW GROUP PLLC, 701 FIFTH AVE, SUITE 6300, SEATTLE, WA, 98104-7092
NUMBER OF CLAIMS: 17
EXEMPLARY CLAIM: 1
LINE COUNT: 14253
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L8 ANSWER 3 OF 4 USPATFULL on STN
TI Compositions and methods for the therapy and diagnosis of colon cancer
AB Compositions and methods for the therapy and diagnosis of cancer, particularly colon cancer, are disclosed. Illustrative compositions comprise one or more colon tumor polypeptides, immunogenic portions thereof, polynucleotides that encode such polypeptides, antigen presenting cell that expresses such polypeptides, and T cells that are specific for cells expressing such polypeptides. The disclosed compositions are useful, for example, in the diagnosis, prevention and/or treatment of diseases, particularly colon cancer.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.
ACCESSION NUMBER: 2002:272801 USPATFULL
TITLE: Compositions and methods for the therapy and diagnosis of colon cancer
INVENTOR(S): Stolk, John A., Bothell, WA, UNITED STATES
Xu, Jiangchun, Bellevue, WA, UNITED STATES
Chenault, Ruth A., Seattle, WA, UNITED STATES
Meagher, Madeleine Joy, Seattle, WA, UNITED STATES
PATENT ASSIGNEE(S): Corixa Corporation, Seattle, WA, UNITED STATES, 98104 (U.S. corporation)

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 2002150922	A1	20021017
APPLICATION INFO.:	US 2001-998598	A1	20011116 (9)

	NUMBER	DATE
PRIORITY INFORMATION:	US 2001-304037P	20010710 (60)
	US 2001-279670P	20010328 (60)
	US 2001-267011P	20010206 (60)
	US 2000-252222P	20001120 (60)

DOCUMENT TYPE: Utility
FILE SEGMENT: APPLICATION
LEGAL REPRESENTATIVE: SEED INTELLECTUAL PROPERTY LAW GROUP PLLC, 701 FIFTH AVE, SUITE 6300, SEATTLE, WA, 98104-7092
NUMBER OF CLAIMS: 17
EXEMPLARY CLAIM: 1
LINE COUNT: 9233
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L8 ANSWER 4 OF 4 USPATFULL on STN
TI Compositions and methods for the therapy and diagnosis of ovarian cancer
AB Compositions and methods for the therapy and diagnosis of cancer, particularly ovarian cancer, are disclosed. Illustrative compositions comprise one or more ovarian tumor polypeptides, immunogenic portions thereof, polynucleotides that encode such polypeptides, antigen presenting cell that expresses such polypeptides, and T cells that are specific for cells expressing such polypeptides. The disclosed compositions are useful, for example, in the diagnosis, prevention and/or treatment of diseases, particularly ovarian cancer.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

ACCESSION NUMBER:	2002:243051 USPATFULL		
TITLE:	Compositions and methods for the therapy and diagnosis of ovarian cancer		
INVENTOR(S):	Algate, Paul A., Issaquah, WA, UNITED STATES Jones, Robert, Seattle, WA, UNITED STATES Harlocker, Susan L., Seattle, WA, UNITED STATES		
PATENT ASSIGNEE(S):	Corixa Corporation, Seattle, WA, UNITED STATES, 98104 (U.S. corporation)		
	NUMBER	KIND	DATE
PATENT INFORMATION:	US 2002132237	A1	20020919
APPLICATION INFO.:	US 2001-867701	A1	20010529 (9)
	NUMBER		DATE
PRIORITY INFORMATION:	US 2000-207484P		20000526 (60)
DOCUMENT TYPE:	Utility		
FILE SEGMENT:	APPLICATION		
LEGAL REPRESENTATIVE:	SEED INTELLECTUAL PROPERTY LAW GROUP PLLC, 701 FIFTH AVE, SUITE 6300, SEATTLE, WA, 98104-7092		
NUMBER OF CLAIMS:	11		
EXEMPLARY CLAIM:	1		
LINE COUNT:	25718		

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

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FILE 'MEDLINE, USPATFULL, DGENE, EMBASE, FSTA, WPIDS, JICST-EPLUS, BIOSIS' ENTERED AT 15:57:27 ON 08 DEC 2003

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L1         12334 S CELL PRODUCTION
L2           184 S CELL PRODUCTION () METHOD
L3           184 S L2 AND L1
L4            14 S L3 AND HUMAN
L5          6547 S L1 AND HUMAN
L6            20 S L3 AND VECTOR
L7          1453 S L5 AND VECTOR
L8             4 S L6 AND PROMOTER
L9          1016 S L7 AND PROMOTER

=> s l9 and factor VIII
L10          137 L9 AND FACTOR VIII

=> s 293S cells
L11          206 293S CELLS

=> s HKB11 cells
L12           12 HKB11 CELLS
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=> s l11 and l9
L13          42 L11 AND L9

=> s l12 and l9
L14           0 L12 AND L9

=> d l13 ti abs ibib 1-15

L13  ANSWER 1 OF 42  USPATFULL on STN
TI     Secreted alpha-helical protein - 32
AB     The present invention relates to polynucleotide and polypeptide
       molecules for mammalian secreted alpha helical protein-32 (Zalpha32).
       The polypeptides, and polynucleotides encoding them, are hormonal and
       may be used to regulate the functioning of the immune system. The
       present invention also includes antibodies to the Zalpha32 polypeptides.

ACCESSION NUMBER:        2003:294776  USPATFULL
TITLE:                   Secreted alpha-helical protein - 32
INVENTOR(S):             Conklin, Darrell C., Seattle, WA, UNITED STATES
                         Gao, Zeren, Redmond, WA, UNITED STATES

                              NUMBER        KIND    DATE
                         ---------------- ------ --------
PATENT INFORMATION:      US 2003207793      A1   20031106
APPLICATION INFO.:       US 2003-461093     A1   20030613  (10)
RELATED APPLN. INFO.:    Continuation of Ser. No. US 2000-578298, filed on 25
                         May 2000, ABANDONED

                               NUMBER         DATE
                         ------------------ --------
PRIORITY INFORMATION:    US 1999-135881P    19990526 (60)
DOCUMENT TYPE:           Utility
FILE SEGMENT:            APPLICATION
LEGAL REPRESENTATIVE:    Gary E. Parker, Patent Department, ZymoGenetics, Inc.,
                         1201 Eastlake Avenue East, Seattle, WA, 98102
NUMBER OF CLAIMS:        8
EXEMPLARY CLAIM:         1
LINE COUNT:              2376

L13  ANSWER 2 OF 42  USPATFULL on STN
TI     Secreted salivary zsig32 polypeptides
AB     The present invention relates to polynucleotide and polypeptide
       molecules for secreted salivary zsig32 polypeptides. The polypeptides,
       and polynucleotides encoding them modulate adhesion or modulate or
       indicate salivary gland function. The present invention also includes
       antibodies and binding proteins for the zsig32 polypeptides.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.
ACCESSION NUMBER:        2003:251856  USPATFULL
TITLE:                   Secreted salivary zsig32 polypeptides
INVENTOR(S):             Sheppard, Paul O., Granite Falls, WA, UNITED STATES
PATENT ASSIGNEE(S):      ZymoGenetics, Inc., Seattle, WA (U.S. corporation)

                              NUMBER        KIND    DATE
                         ---------------- ------ --------
PATENT INFORMATION:      US 2003176645      A1   20030918
APPLICATION INFO.:       US 2002-82649      A1   20020222  (10)
RELATED APPLN. INFO.:    Continuation of Ser. No. US 1999-442952, filed on 18
                         Nov 1999, ABANDONED Continuation of Ser. No. US
                         1998-81180, filed on 19 May 1998, GRANTED, Pat. No. US
                         6022847

                               NUMBER         DATE
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PRIORITY INFORMATION:	US 1997-41263P	19970319 (60)
DOCUMENT TYPE:	Utility	
FILE SEGMENT:	APPLICATION	
LEGAL REPRESENTATIVE:	Brian J. Walsh, Patent Department, ZymoGenetics, Inc., 1201 Eastlake Avenue East, Seattle, WA, 98102	
NUMBER OF CLAIMS:	33	
EXEMPLARY CLAIM:	1	
NUMBER OF DRAWINGS:	1 Drawing Page(s)	
LINE COUNT:	3068	

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L13 ANSWER 3 OF 42 USPATFULL on STN

TI Beta-1,3-galactosyltransferase homologs

AB The present invention relates to polynucleotide and polypeptide molecules for znssp2, a novel member of the galactosyltransferase family. The polypeptides, and polynucleotides encoding them, are cell-cell interaction and glycoprotein synthesis modulating and may be used for delivery and therapeutics. The present invention also includes antibodies to the znssp2 polypeptides.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

ACCESSION NUMBER:	2003:251136 USPATFULL		
TITLE:	Beta-1,3-galactosyltransferase homologs		
INVENTOR(S):	Conklin, Darrell C., Seattle, WA, UNITED STATES Yamamoto, Gayle, Seattle, WA, UNITED STATES Jaspers, Stephen R., Edmonds, WA, UNITED STATES Gao, Zeren, Redmond, WA, UNITED STATES		
PATENT ASSIGNEE(S):	ZymoGenetics, Inc. (U.S. corporation)		
	NUMBER	KIND	DATE
PATENT INFORMATION:	US 2003175922	A1	20030918
APPLICATION INFO.:	US 2002-177079	A1	20020621 (10)
RELATED APPLN. INFO.:	Division of Ser. No. US 1999-459133, filed on 10 Dec 1999, GRANTED, Pat. No. US 6416988		
	NUMBER		DATE
PRIORITY INFORMATION:	US 1998-111697P		19981210 (60)
DOCUMENT TYPE:	Utility		
FILE SEGMENT:	APPLICATION		
LEGAL REPRESENTATIVE:	Robyn Adams, Patent Department, ZymoGenetics, Inc., 1201 Eastlake Avenue East, Seattle, WA, 98102		
NUMBER OF CLAIMS:	24		
EXEMPLARY CLAIM:	1		
LINE COUNT:	3851		

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L13 ANSWER 4 OF 42 USPATFULL on STN

TI Mammalian adhesion protease peptides

AB The present invention relates to polynucleotide and polypeptide molecules, and variants thereof, for MAPP, a novel member of the Disintegrin Proteases. The polypeptides, and polynucleotides encoding them, are cell-cell interaction modulating and may be used for delivery and therapeutics. The present invention also includes antibodies to the MAPP polypeptides.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

ACCESSION NUMBER:	2003:250477 USPATFULL
TITLE:	Mammalian adhesion protease peptides
INVENTOR(S):	Sheppard, Paul O., Granite Falls, WA, UNITED STATES Baindur, Nand, Edmonds, WA, UNITED STATES Bishop, Paul D., Fall City, WA, UNITED STATES

PATENT ASSIGNEE(S): ZymoGenetics, Inc. (U.S. corporation)

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 2003175262	A1	20030918
APPLICATION INFO.:	US 2002-177308	A1	20020621 (10)

RELATED APPLN. INFO.: Division of Ser. No. US 2000-632098, filed on 2 Aug 2000, GRANTED, Pat. No. US 6420154

	NUMBER	DATE
PRIORITY INFORMATION:	US 1999-146968P	19990803 (60)

DOCUMENT TYPE: Utility
FILE SEGMENT: APPLICATION
LEGAL REPRESENTATIVE: Robyn Adams, ZymoGenetics, Inc., Patent Department, 1201 Eastlake Avenue East, Seattle, WA, 98102
NUMBER OF CLAIMS: 19
EXEMPLARY CLAIM: 1
LINE COUNT: 3811
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L13 ANSWER 5 OF 42 USPATFULL on STN
TI Adipocyte-specific protein homologs
AB The present invention relates to polynucleotide and polypeptide molecules for zsig39, a novel member of the family of proteins bearing a collagen-like domain and a globular domain. The polypeptides, and polynucleotides encoding them, are involved in dimerization or oligomerization and may be used in the study thereof. The present invention also includes antibodies to the zsig39 polypeptides.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.
ACCESSION NUMBER: 2003:245133 USPATFULL
TITLE: Adipocyte-specific protein homologs
INVENTOR(S): Sheppard, Paul O., Redmond, WA, UNITED STATES
Humes, Jacqueline M., Seattle, WA, UNITED STATES
PATENT ASSIGNEE(S): ZymoGenetics, Inc. (U.S. corporation)

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 2003171547	A1	20030911
APPLICATION INFO.:	US 2002-197293	A1	20020716 (10)

RELATED APPLN. INFO.: Continuation of Ser. No. US 2000-686838, filed on 10 Oct 2000, GRANTED, Pat. No. US 6482612 Division of Ser. No. US 1998-140804, filed on 26 Aug 1998, GRANTED, Pat. No. US 6197930

	NUMBER	DATE
PRIORITY INFORMATION:	US 1997-56983P	19970826 (60)

DOCUMENT TYPE: Utility
FILE SEGMENT: APPLICATION
LEGAL REPRESENTATIVE: Brian J. Walsh, ZymoGenetics, Inc., Patent Department, 1201 Eastlake Avenue East, Seattle, WA, 98102
NUMBER OF CLAIMS: 37
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 2 Drawing Page(s)
LINE COUNT: 3818
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L13 ANSWER 6 OF 42 USPATFULL on STN
TI Mammalian secretory peptide - 9
AB Novel mammalian secretory polypeptides, polynucleotides encoding the polypeptides (called Zsig9), and related compositions and methods including antibodies and anti-idiotypic antibodies. Overexpression of

these proteins is indicative of the presence of cancer. Antibodies and anti-sense nucleotides can be used therapeutically to treat the disease. Furthermore, antibodies to Zsig9 and nucleotide primers and probes can be used to diagnose the presence of tumors.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

ACCESSION NUMBER:	2003:245128 USPATFULL
TITLE:	Mammalian secretory peptide - 9
INVENTOR(S):	Sheppard, Paul O., Granite Falls, WA, UNITED STATES Jelinek, Laura J., Seattle, WA, UNITED STATES
PATENT ASSIGNEE(S):	ZymoGenetics, Inc. (U.S. corporation)

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 2003171542	A1	20030911
APPLICATION INFO.:	US 2001-82502	A1	20011019 (10)

RELATED APPLN. INFO.: Continuation of Ser. No. US 1999-318028, filed on 25 May 1999, ABANDONED Continuation-in-part of Ser. No. US 1998-109808, filed on 2 Jul 1998, ABANDONED

	NUMBER	DATE
PRIORITY INFORMATION:	US 1998-89899P	19980617 (60)
	US 1998-85983P	19980519 (60)
	US 1997-51704P	19970703 (60)

DOCUMENT TYPE:	Utility
FILE SEGMENT:	APPLICATION
LEGAL REPRESENTATIVE:	Shelby J Walker, ZymoGenetics, Inc., 1201 Eastlake Avenue East, Seattle, WA, 98102
NUMBER OF CLAIMS:	15
EXEMPLARY CLAIM:	1
LINE COUNT:	2092

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L13 ANSWER 7 OF 42 USPATFULL on STN

TI Disintegrin homologue, MAHBP

AB The present invention relates to polynucleotide and polypeptide molecules for MAHBP, a novel member of the Disintegrin Proteases. The polypeptides, and polynucleotides encoding them, are believed to be cell-cell interaction modulating and may be used for delivery and therapeutics. The present invention also includes antibodies to the MAHBP polypeptides.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

ACCESSION NUMBER:	2003:219774 USPATFULL
TITLE:	Disintegrin homologue, MAHBP
INVENTOR(S):	Sheppard, Paul O., Granite Falls, WA, UNITED STATES Baindur, Nand, Edmonds, WA, UNITED STATES Deisher, Theresa A., Seattle, WA, UNITED STATES Bishop, Paul D., Fall City, WA, UNITED STATES Taft, David W., Seattle, WA, UNITED STATES
PATENT ASSIGNEE(S):	ZymoGenetics, Inc. (U.S. corporation)

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 2003153064	A1	20030814
APPLICATION INFO.:	US 2002-260506	A1	20020927 (10)

RELATED APPLN. INFO.: Division of Ser. No. US 2000-631534, filed on 3 Aug 2000, ABANDONED

	NUMBER	DATE
PRIORITY INFORMATION:	US 1999-147410P	19990805 (60)

DOCUMENT TYPE: Utility

FILE SEGMENT: APPLICATION
LEGAL REPRESENTATIVE: Robyn Adams, ZymoGenetics, Inc., Patent Department, 1201 Eastlake Avenue East, Seattle, WA, 98102
NUMBER OF CLAIMS: 31
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 17 Drawing Page(s)
LINE COUNT: 3895
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L13 ANSWER 8 OF 42 USPATFULL on STN
TI Secreted protein, ZTNF9
AB Novel tumor necrosis factor ligand polypeptides, polynucleotides encoding the polypeptides, and related compositions and methods are disclosed. The polypeptides may be used within methods relating to immune response, and may also be used in the development of immuno-regulatory therapeutics. Also provided are antibodies, binding proteins, agonists and antagonists of the ligand polypeptides.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.
ACCESSION NUMBER: 2003:213828 USPATFULL
TITLE: Secreted protein, ZTNF9
INVENTOR(S): Fox, Brian A., Seattle, WA, UNITED STATES
Gross, Jane A., Seattle, WA, UNITED STATES

	NUMBER	KIND	DATE	
PATENT INFORMATION:	US 2003148466	A1	20030807	
APPLICATION INFO.:	US 2002-273231	A1	20021016	(10)

	NUMBER	DATE
PRIORITY INFORMATION:	US 2001-329931P	20011017 (60)

DOCUMENT TYPE: Utility
FILE SEGMENT: APPLICATION
LEGAL REPRESENTATIVE: Robyn Adams, ZymoGenetics, Inc, 1201 Eastlake Avenue East, Seattle, WA, 98102
NUMBER OF CLAIMS: 30
EXEMPLARY CLAIM: 1
LINE COUNT: 2762
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L13 ANSWER 9 OF 42 USPATFULL on STN
TI Protease-activated receptor PAR4 (ZCHEMR2)
AB The present invention relates to polynucleotide and polypeptide molecules for PAR4, a novel member of the protease-activated receptor family. The polypeptides, and polynucleotides encoding them, mediate biological responses and/or cellular signaling in response to proteases. Protease cleavage of PAR4 exposes a PAR4 extracellular amino terminal portion that serves as a ligand for the PAR4 receptor. PAR4 may be used as a target in drug screening, and further used to identify proteinaceous or non-proteinaceous PAR4 agonists and antagonists. The present invention also includes antibodies to the PAR4 polypeptides.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.
ACCESSION NUMBER: 2003:206861 USPATFULL
TITLE: Protease-activated receptor PAR4 (ZCHEMR2)
INVENTOR(S): Xu, Wenfeng, Mukilteo, WA, UNITED STATES
Presnell, Scott R., Tacoma, WA, UNITED STATES
Yee, David P., Cambridge, MA, UNITED STATES
Foster, Donald C., Lake Forest Park, WA, UNITED STATES

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 2003143218	A1	20030731

APPLICATION INFO.: US 2002-187049 A1 20020628 (10)
RELATED APPLN. INFO.: Division of Ser. No. US 2000-479130, filed on 7 Jan 2000, GRANTED, Pat. No. US 6436400 Division of Ser. No. US 2000-480720, filed on 7 Jan 2000, ABANDONED Division of Ser. No. US 1999-371333, filed on 10 Aug 1999, PENDING Continuation of Ser. No. US 1998-53866, filed on 1 Apr 1998, GRANTED, Pat. No. US 6111075

	NUMBER	DATE
PRIORITY INFORMATION:	WO 1999-US7100	19990331

DOCUMENT TYPE: Utility
FILE SEGMENT: APPLICATION
LEGAL REPRESENTATIVE: Phillip B.C. Jones, J.D., Ph.D., ZymoGenetics, Inc., Patent Department, 1201 Eastlake Avenue East, Seattle, WA, 98102
NUMBER OF CLAIMS: 11
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 1 Drawing Page(s)
LINE COUNT: 2307
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L13 ANSWER 10 OF 42 USPATFULL on STN
TI Galactosyltransferase homolog, ZNSSP11
AB Novel soluble, and membrane bound, Beta1,3 galactosyltransferase polypeptides, polynucleotides encoding the polypeptides, antibodies and related compositions and methods are disclosed. The polypeptides may be used for detecting anti-complementary molecules, agonists and antagonists. The polypeptides, polynucleotides and antibodies may also be used in methods that modulate cell-cell interactions, and glycoprotein and glycolipid modifications.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.
ACCESSION NUMBER: 2003:152732 USPATFULL
TITLE: Galactosyltransferase homolog, ZNSSP11
INVENTOR(S): Adler, David A., Bainbridge Island, WA, UNITED STATES
Holloway, James L., Seattle, WA, UNITED STATES
Yamamoto, Gayle, Seattle, WA, UNITED STATES

	NUMBER	KIND	DATE	
PATENT INFORMATION:	US 2003104398	A1	20030605	
APPLICATION INFO.:	US 2001-15725	A1	20011102	(10)

	NUMBER	DATE
PRIORITY INFORMATION:	US 2000-245516P	20001103 (60)

DOCUMENT TYPE: Utility
FILE SEGMENT: APPLICATION
LEGAL REPRESENTATIVE: Robyn Adams, ZymoGenetics, Inc., 1201 Eastlake Avenue East, Seattle, WA, 98102
NUMBER OF CLAIMS: 20
EXEMPLARY CLAIM: 1
LINE COUNT: 3225
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L13 ANSWER 11 OF 42 USPATFULL on STN
TI Disintegrin homolog, ZSNK16
AB The present invention relates to polynucleotide and polypeptide molecules, and variants thereof, for ZSNK16, novel members of the Disintegrin Proteases. The polypeptides, and polynucleotides encoding them, are cell-cell interaction modulating and may be used for delivery and therapeutics. The present invention also includes antibodies to the ZSNK16 polypeptides.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.
ACCESSION NUMBER: 2003:140552 USPATFULL
TITLE: Disintegrin homolog, ZSNK16
INVENTOR(S): Fox, Brian A., Seattle, WA, UNITED STATES
Sheppard, Paul O., Granite Falls, WA, UNITED STATES

	NUMBER	KIND	DATE	
PATENT INFORMATION:	US 2003096393	A1	20030522	
APPLICATION INFO.:	US 2002-78866	A1	20020220	(10)

	NUMBER	DATE
PRIORITY INFORMATION:	US 2001-270276P	20010220 (60)

DOCUMENT TYPE: Utility
FILE SEGMENT: APPLICATION
LEGAL REPRESENTATIVE: Robyn Adams, ZymoGenetics, Inc., 1201 Eastlake Avenue East, Seattle, WA, 98102
NUMBER OF CLAIMS: 24
EXEMPLARY CLAIM: 1
LINE COUNT: 2712
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L13 ANSWER 12 OF 42 USPATFULL on STN
TI Methods for treating patients with adenoviral vectors
AB The present invention addresses the need to improve the yields of viral vectors when grown in cell culture systems. In particular, it has been demonstrated that for adenovirus, the use of low-medium perfusion rates in an attached cell culture system provides for improved yields. In other embodiments, the inventors have shown that there is improved Ad-p53 production witrh cells grown in serum-free conditions, and in particular in serum-free suspension culture. Also important to the increase of yields is the use of detergent lysis. Combination of these aspects of the invention permits purification of virus by a single chromatography step that results in purified virus of the same quality as preparations from double CsCl banding using an ultracentrifuge.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.
ACCESSION NUMBER: 2003:10680 USPATFULL
TITLE: Methods for treating patients with adenoviral vectors
INVENTOR(S): Zhang, Shuyuan, Sugar Land, TX, UNITED STATES
Thwin, Capucine, Spring, TX, UNITED STATES
Wu, Zheng, Sugar Land, TX, UNITED STATES
Cho, Toohyon, Houston, TX, UNITED STATES
PATENT ASSIGNEE(S): Introgen Therapeutics, Inc. (U.S. corporation)

	NUMBER	KIND	DATE	
PATENT INFORMATION:	US 2003008375	A1	20030109	
APPLICATION INFO.:	US 2001-33491	A1	20011227	(10)

RELATED APPLN. INFO.: Continuation of Ser. No. US 2000-556570, filed on 24 Apr 2000, PENDING Continuation of Ser. No. US 1997-975519, filed on 20 Nov 1997, GRANTED, Pat. No. US 6194191

	NUMBER	DATE
PRIORITY INFORMATION:	US 1996-31329P	19961120 (60)

DOCUMENT TYPE: Utility
FILE SEGMENT: APPLICATION
LEGAL REPRESENTATIVE: MARSHALL, GERSTEIN & BORUN, 6300 SEARS TOWER, 233 SOUTH WACKER, CHICAGO, IL, 60606-6357
NUMBER OF CLAIMS: 69

EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 42 Drawing Page(s)
LINE COUNT: 3730
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L13 ANSWER 13 OF 42 USPATFULL on STN
TI AN IMPROVED METHOD FOR THE PRODUCTION AND PURIFICATION OF ADENOVIRAL VECTORS
AB The present invention addresses the need to improve the yields of viral vectors when grown in cell culture systems. In particular, it has been demonstrated that for adenovirus, the use of low-medium perfusion rates in an attached cell culture system provides for improved yields. In other embodiments, the inventors have shown that there is improved Ad-p53 production witrh cells grown in serum-free conditions, and in particular in serum-free suspension culture. Also important to the increase of yields is the use of detergent lysis. Combination of these aspects of the invention permits purification of virus by a single chromatography step that results in purified virus of the same quality as preparations from double CsCl banding using an ultracentrifuge.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.
ACCESSION NUMBER: 2002:322559 USPATFULL
TITLE: AN IMPROVED METHOD FOR THE PRODUCTION AND PURIFICATION OF ADENOVIRAL VECTORS
INVENTOR(S): Zhang, Shuyuan, Sugar Land, TX, UNITED STATES
Thwin, Capucine, Spring, TX, UNITED STATES
Wu, Zheng, Sugar Land, TX, UNITED STATES
Cho, Toohyon, UNITED STATES
Gallagher, Shawn, Missouri City, TX, UNITED STATES
PATENT ASSIGNEE(S): Introgen Therapeutics, Inc. (U.S. corporation)

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 2002182723	A1	20021205
APPLICATION INFO.:	US 2001-880609	A1	20010612 (9)

RELATED APPLN. INFO.: Division of Ser. No. US 1998-203078, filed on 1 Dec 1998, PENDING Continuation-in-part of Ser. No. US 1997-975519, filed on 20 Nov 1997, GRANTED, Pat. No. US 6194191

	NUMBER	DATE
PRIORITY INFORMATION:	US 1996-31329P	19961120 (60)

DOCUMENT TYPE: Utility
FILE SEGMENT: APPLICATION
LEGAL REPRESENTATIVE: Steven L. Highlander, FULBRIGHT & JAWORSKI L.L.P., Suite 2400, 600 Congress Avenue, Austin, TX, 78701
NUMBER OF CLAIMS: 43
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 49 Drawing Page(s)
LINE COUNT: 6000
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L13 ANSWER 14 OF 42 USPATFULL on STN
TI Methods for producing purified adenoviral vectors
AB The present invention addresses the need to improve the yields of viral vectors when grown in cell culture systems. In particular, it has been demonstrated that for adenovirus, the use of low-medium perfusion rates in an attached cell culture system provides for improved yields. In other embodiments, the inventors have shown that there is improved Ad-p53 production with cells grown in serum-free conditions, and in particular in serum-free suspension culture. Also important to the increase of yields is the use of detergent lysis. Combination of these aspects of the invention permits purification of virus by a single

chromatography step that results in purified virus of the same quality as preparations from double CsCl banding using an ultracentrifuge.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

ACCESSION NUMBER:	2002:314738 USPATFULL
TITLE:	Methods for producing purified adenoviral vectors
INVENTOR(S):	Zhang, Shuyuan, Sugarland, TX, UNITED STATES Thwin, Capucine, Spring, TX, UNITED STATES Wu, Zheng, Sugarland, TX, UNITED STATES Cho, Toohyon, Houston, TX, UNITED STATES
PATENT ASSIGNEE(S):	Introgen Therapeutics, Inc. (U.S. corporation)

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 2002177215	A1	20021128
APPLICATION INFO.:	US 2001-33571	A1	20011227 (10)

RELATED APPLN. INFO.: Continuation of Ser. No. US 2000-556570, filed on 24 Apr 2000, PENDING Continuation of Ser. No. US 1997-975519, filed on 20 Nov 1997, GRANTED, Pat. No. US 6194191

	NUMBER	DATE
PRIORITY INFORMATION:	US 1996-31329P	19961120 (60)

DOCUMENT TYPE:	Utility
FILE SEGMENT:	APPLICATION
LEGAL REPRESENTATIVE:	MARSHALL, GERSTEIN & BORUN, 6300 SEARS TOWER, 233 SOUTH WACKER, CHICAGO, IL, 60606-6357
NUMBER OF CLAIMS:	69
EXEMPLARY CLAIM:	1
NUMBER OF DRAWINGS:	32 Drawing Page(s)
LINE COUNT:	3720

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L13 ANSWER 15 OF 42 USPATFULL on STN

TI Secreted proteins encoded by **human** chromosome 13

AB The present invention relates to polynucleotide and polypeptide molecules a for zsig46 polypeptide, a novel secreted protein located on **human** chromosome 13. The zsig46 polypeptides, and polynucleotides encoding them, are secreted proteins and may be used in the study of receptors for which a ligand has not yet been identified, of secretory pathways and the like. The present invention also includes antibodies to the zsig46 polypeptides.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

ACCESSION NUMBER:	2002:308493 USPATFULL
TITLE:	Secreted proteins encoded by **human** chromosome 13
INVENTOR(S):	Sheppard, Paul O., Granite Falls, WA, UNITED STATES Gilbertson, Debra G., Seattle, WA, UNITED STATES
PATENT ASSIGNEE(S):	ZymoGenetics, Inc. (U.S. corporation)

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 2002173624	A1	20021121
APPLICATION INFO.:	US 2001-10050	A1	20011109 (10)

RELATED APPLN. INFO.: Continuation of Ser. No. US 1998-122383, filed on 24 Jul 1998, PENDING

	NUMBER	DATE
PRIORITY INFORMATION:	US 1997-53613P	19970724 (60)

DOCUMENT TYPE:	Utility
FILE SEGMENT:	APPLICATION

LEGAL REPRESENTATIVE: Brian J. Walsh, ZymoGenetics, Inc., 1201 Eastlake Avenue East, Seattle, WA, 98102
NUMBER OF CLAIMS: 33
EXEMPLARY CLAIM: 1
LINE COUNT: 2798
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

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E1	1		CHO ZUIKO/AU
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E4	1		CHOA A J O/AU
E5	1		CHOA A K/AU
E6	1		CHOA A K H/AU
E7	3		CHOA B/AU
E8	4		CHOA B H G/AU
E9	2		CHOA C/AU
E10	1		CHOA C G/AU
E11	2		CHOA C H/AU
E12	1		CHOA CHING GUAN/AU

=> e chan,S/au

E1	3		CHAN ZUI NGA/AU
E2	1		CHAN ZUNYU/AU
E3	0	-->	CHAN,S/AU
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E5	1		CHANA A K/AU
E6	1		CHANA A S/AU
E7	2		CHANA ANTONIA/AU
E8	4		CHANA ANTONIO/AU
E9	3		CHANA B S/AU
E10	2		CHANA C/AU
E11	2		CHANA C H/AU
E12	2		CHANA CUEVAS P/AU

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L8	pCIS25DTR	3	L8
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L5	l2 and L4	426002	L5
L4	L3 and l1	426002	L4
L3	HKB11 cells	426002	L3
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L1	cell production adj2 factor VIII	515096	L1

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L5: Entry 1 of 1 File: USPT Sep 14, 1999

DOCUMENT-IDENTIFIER: US 5952198 A
TITLE: Production of recombinant Factor VIII in the presence of liposome-like substances of mixed composition

Abstract Text (1):
Recombinant Factor VIII expression in a mammalian cell culture can be increased by including a novel liposome-like substance in the culture medium. The liposome-like substance comprises at least two (preferably at least three) different lipids in defined molar ratios. In a preferred embodiment, the addition of a liposome-like substance comprised of dioleoyl phosphatidylcholine, phosphatidylethanolamine, and phosphatidylserine in a molar ratio of 4:1:1 to the culture medium of GS-MDR cells resulted in an increase in FVIII production by a factor greater than five.

US Patent No. (1):
5952198

Brief Summary Text (3):
This disclosure is concerned generally with the production of recombinant Factor VIII in a mammalian cell expression system. Specifically, the disclosure relates to the addition of a liposome-like substance containing lipids in defined ratios to the mammalian cell culture medium to increase yields of recombinant Factor VIII.

Brief Summary Text (5):
Factor Vil is a plasma protein required for normal hemostasis, or clotting of the blood. Functional Factor VIII is lacking in individuals with hemophilia A because of a mutation in the gene encoding this protein, which is located in the X-chromosome. To control bleeding episodes, hemophiliacs must be treated with Factor VIII, which historically has been isolated from human blood plasma.

Brief Summary Text (6):
The human Factor VIII gene encompasses 186,000 base pairs and constitutes 0.1% of the entire X-chromosome, making it among the largest genes known (1). The transcription product of this gene, which is derived from 26 exons, is a messenger RNA molecule of .about.9000 bases in length, coding for a large protein of 2351 amino acids. Structural studies of Factor VIII indicate that it is a glycoprotein, containing a significant number of carbohydrate residues. The cDNA coding for Factor VIII has been cloned (2,3) and stably expressed in baby hamster kidney cells (BHK-21) (3) and Chinese hamster ovary cells (4). The availability of these high producing cell clones has made large-scale production of recombinant Factor VIII (rFVIII) feasible. Two significant challenges in the commercial production of rFVIII are (i) the development of a serumfree medium that will support high density cultures and stabilize rFVIII, and (ii) an efficient purification scheme that will yield high purity rFVIII.

Brief Summary Text (7):
Previously it has been demonstrated that the addition of bovine lipoprotein or human low density lipoprotein to serumfree cultures significantly improve the productivity of recombinant BHK-21 and human embryonic kidney (293S) cells expressing rFVIII (5). The co-expression of vonWillebrand factor and the addition of phospholipids to serumfree medium have been shown to be effective in enhancing the stability of rFVIII produced by rFVIII expressing CHO cells (6).

Brief Summary Text (10):
I have found a method and medium for substantially increasing the productivity of a mammalian cell expression system producing recombinant Factor VIII by a factor greater than about four. The essential step of the method consists of the addition of a liposome-like substance to the cell growth medium of the expression system. As used herein, "liposome-like substance" means vesicles or other tertiary-structures comprising one or more bilayers comprised of at least two different lipids in fixed molar ratios. Molar ratios should be understood to be approximate.

Detailed Description Text (6):
GS-10 (recombinant 293S cells expressing high levels of Factor VIII) cells were maintained as serumfree cultures in shake flasks using a serumfree medium (Dulbecco's minimum essential medium and F12 at a ratio of 1:1, obtained from Life Technologies, Bethesda, Md.) supplemented with insulin (10.mu.g/ml) and transferrin (25 .mu.g/ml). Long term evaluation was done in shake flasks with an initial seeding density of 3.times.10.sup.6 cells/ml. Complete medium exchanges were done at 24-hour intervals where cells were spun, washed and reseeded at 3.times.10.sup.6 cells/ml. A typical shaker culture contains 25-50 ml of cells. Factor VIII activity was determined by Coatest VIII (Kabi Pharmacia, Franklin, Ohio), a chromogenic assay, according to manufacturer's instructions.

Detailed Description Text (7):
The initial screening of phospholipids was done using 24-hour plate cultures. After determining the optimal ratio of various phospholipids, the study was then confirmed in shake flasks over a period of 10-14 days. As shown in Table 1, while PC and PE alone had no effect on Factor VIII expression, PS alone was found to be inhibitory. By combining PC, PS, and PE at various ratios, significant increases in Factor VIII expression were observed. The highest productivity was observed in cells supplemented with PC:PE:PS (4:1:1), PC:PS:cholesterol (8:1:1), and PC:PS:palmitic acid:linoleic acid (7:3:0.5:0.5). The optimal concentration of phospholipids was found to be 30 .mu.g/ml. The optimal length of the acyl side chain of various phospholipids was determined to be C18. All optimization studies were subsequently done with dioleoyl phospholipids.

Detailed Description Text (9):
Expression of factor VIII in continuous culture

Detailed Description Text (10):
I measured the effect of various liposome-like substances on the production of factor VIII in long term shake flask cultures with PC:PE:PS (4:1:1), PC:PS: palmitic acid:linoleic acid (7:3:0.5:0.5), and PC:PS:cholesterol (8:1:1). The culture conditions were done as described in Example I. The concentration of the liposome-like substances was at 30 .mu.g/ml. Complete medium exchange was done at 24-hour intervals. Results are shown in Table 2.

Detailed Description Text (12):
Expression of Factor Vil in continuous cultures

Detailed Description Text (13):
The effect of various liposomes on the production of truncated Factor VIII (deletion of all or part of the B domain of Factor VIII) was examined in recombinant 293S cells expressing high levels of a B-domain-deleted Factor VIII with the following sequence (SEQ ID NO. 1) joining the 90-kD and 80-kD fragments of Factor VIII:

Detailed Description Text (14):
"90kD--Ser-Phe-Ser-Gln-Asn-Pro-Pro-Val-Leu-Lys-Arg-His-Gln-Arg--80kD (SEQ ID NO:1)". (Amino acid abbreviations are as given in Ref. (7), incorporated herein by reference.) This truncated Factor VIII is essentially as described in Ref. (8), incorporated herein by reference. The culture conditions were done in 12-well plates with an initial seeding density of 2.times.105 cells per well in DMEM/F12 (1:1) supplemented with 5% fetal bovine serum. After confluency was reached the cells were washed with PBS and fed with the serumfree production as described in Example I. Results are shown in Table 3, where at least a threefold increase in productivity over the saline control is shown. The highest productivites were observed in cells

supplemented with dioleoyl PC/PE/PS (8:1:1) and dioleoyl PC/PS/cholesterol (8:1:1).

Detailed Description Text (16):
We have demonstrated that lipid mixtures, when delivered in the form of liposome-like substances, significantly enhance the production of Factor VIII (in both full length and truncated forms) in recombinant cells. These liposome-like substances can be used as medium supplements to support production of Factor VIII, preferably continuous production of Factor VIII. As used herein, the term Factor VIII is intended to include all variants or truncated forms of Factor VIII having Factor VIII activity.

Detailed Description Paragraph Table (1):
TABLE 1 Effect of phospholipid on the expression of Factor VIII in GS-MDR cells Phospholipids FVIII Titer (U/ml) Dioleoyl PC/PS (7:3) 2.8 Dioleoyl PC/PS (8:2) 2.5 Dioleoyl PC/PS (9:1) 1.4 Dioleoyl PC/PS/palmitic/linoleic acid 3.4 (7:3:0.5:0.5) Dioleoyl PC/PE/PS (4:1:1) 3.5 Dioleoyl PC/PE/PS (8:1:1) 1.7 DioleoylPC/PE/PS (16:1:2) 2.1 Dioleoyl PC 0.65 Dioleoyl PE 0.55 Dioleoyl PS 0.15 Saline 0.60

Detailed Description Paragraph Table (2):
TABLE 2 Production of Factor VIII in continuous cultures of GS-10 cells FVIII titer (U/ml) PC/PE/PS PC/PS/pm/ln PC/PS/Cholesterol Days (4:1:1) (7:3:0.5:0.5) (8:1:1) Medium only 1 2.44 2.51 2.41 0.35 2 2.78 2.89 2.72 0.4 3 2.46 2.77 2.69 0.44 4 2.82 2.8 2.7 0.48 5 2.88 2.95 2.99 0.42 6 2.9 3.1 2.95 0.41 7 3.21 3.18 3.12 0.54 8 3.18 3.22 3.06 0.55 9 3.02 3.16 3.14 0.58 10 3.34 3.38 3.27 0.53 11 2.97 3.15 3.19 0.49 12 3.12 2.95 2.98 0.52 13 3.02 3.12 2.71 0.54 14 2.89 3.16 3.22 0.56 15 3.22 3.38 3.34 0.51 pm = palmitic acid ln = linoleic acid

Detailed Description Paragraph Table (3):
TABLE 3 Effect of phospholipid on the expression of a B-domain deleted Factor VIII variant in 293S cells Phospholipids FVIII Titer (U/ml) Dioleoyl PC/PS (7:3) 2.80 Dioleoyl PC/PS (9:1) 0.85 Dioleoyl PC/PS/Cholesterol (8:1:1) 4.0 Dioleoyl PC 1.0 Dioleoyl PC/PE/PS (8:1:1) 3.04 Saline 0.9

CLAIMS:

1. A method for increasing the production level of recombinant factor VIII in a mammalian cell culture expression system by at least fourfold, comprising the step of adding to the culture system a liposome-like substance comprising at least 2 different lipids in a molar ratio under conditions sufficient to assure the fourfold increase in productivity, wherein the lipids in the molar ratio are selected from the group consisting of phosphatidylcholine, phosphatidylethanolamine. and phosphatidylserine in a molar ratio of about 4:1:1, phosphatidylcholine, phosphatidvlserine and cholesterol in a molar ratio of about 8:1:1, and phosphatidylcholine, phosphatidvlserine, palmitic acid, and linoleic acid in a molar ratio of about 7:3:0.5:0.5.

2. The method of claim 1 wherein the recombinant factor VIII is truncated.

3. The method of claim 2 wherein the truncated factor VIII has a 90 kD fragment and an 80 kD fragment which are linked by a polypeptide having a sequence Ser-Phe-Ser-Gln-Asn-Pro-Pro-Val-Leu-Lys-Arg-His-Gln-Arg (SEQ ID NO: 1).

4. A culture medium containing a liposome-like substance, wherein said liposome-like substance comprises at least 2 different lipids in a molar ratio sufficient to assure at least a fourfold increase in factor VIII expression in a mammalian cell culture system, wherein the lipids in the molar ratio are selected from the group consisting of phosphatidylcholine, phosphatidylethanolamine, and phosphatidylserine in a molar ratio of about 4:1:1, phosphatidylcholine, phosphatidylserine and cholesterol in a molar ratio of about 8:1:1, and phosphatidylcholine, phosphatidylserine, palmitic acid, and linoleic acid in a molar ratio of about

7:3:0.5:0.5.

5. The medium of claim 4 wherein the recombinant factor VIII is truncated.

6. The medium of claim 5 wherein the truncated factor VIII has a 90 kD fragment and an 80 kD fragment which are linked by a polypeptide having a sequence Ser-Phe-Ser-Gln-Asn-Pro-Pro-Val-Leu-Lys-Arg-His-Gln-Arg (SEQ ID NO: 1).

7. A method for increasing the production level of a truncated recombinant factor VIII in a mammalian cell culture expression system by at least threefold, comprising the step of adding to the culture system a liposome-like substance under conditions sufficient to assure the threefold increase in productivity, wherein the liposome like substance is comprised of phosphstidylcholine, phosphatidylethanolamine, and phosphatidylserine in a molar ratio of about 8:1:1.